

A Study of the Complications Associated with Haemodialysis Vascular Access in Patients with Renal Failure

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Abstract

Successful haemodialysis depends on the availability of safe, efficient, and durable access to the vascular tree. This can be provided by creation of an arteriovenous fistula, insertion of a synthetic vascular graft or insertion of a central venous catheter. Established haemodialysis vascular access is associated with a number of important complications which can impact significantly on both the quality of life and survival of haemodialysis patients.

The primary aim of this thesis was to perform a detailed evaluation of the risks to health conferred by haemodialysis vascular access and its maintenance in patients with advanced renal failure.

The work described in this thesis describes the relative strength and independence of association between haemodialysis vascular access type and risk of mortality, bacteraemia and catheter thrombosis. Greater clarity is demonstrated on the relative effect of heparin-based haemodialysis catheter lock solutions on markers of systemic coagulation in vivo, whilst the in-vitro variability of antimicrobial activity against planktonic and biofilm-embedded staphylococci achieved with catheter lock solutions containing heparin and vancomycin, alone and in combination, is clearly shown.

New insights are gained into the benefits of contrast magnetic resonance venography as a tool for demonstrating thrombosis and stenosis of the central veins in the assessment of vascular access in haemodialysis patients. Similarly, the emergence of

a new disease, nephrogenic systemic fibrosis was found and its association with gadolinium-enhanced magnetic resonance imaging was explored in detail.

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Declaration

The work presented in this thesis was performed solely by the author with the exception of the work detailed in Chapter 7. This study was formulated in conjunction with Dr Tara Collidge (Renal Unit, Glasgow Royal Infirmary) and Dr Patrick Mark (Renal Unit, Western Infirmary Glasgow) and with whom data collection was conducted in equal measure. All other aspects of this work presented in this thesis were conducted by the author alone. As first author, I presented the results of the study at a meeting of the Scottish Renal Association. As first author, Dr Collidge presented the results of the study at a meeting of the UK Renal Association. A manuscript describing the data has been published in a peer-reviewed journal with Dr Collidge as first author. I have obtained permission from Dr Collidge and Dr Mark to include this work within this thesis.

This thesis has not been submitted or accepted as a previous degree either to the University of Glasgow or elsewhere. Several chapters have been presented at local, national and international meetings as poster presentations and oral presentations. Some chapters have been published in peer-reviewed journals as individual manuscripts.

Where graphs and figures have been used from other publications, appropriate permission has been granted by the copyright holder.

Peter Thomson, August 2009

List of Publications

Vascular access in haemodialysis patients: a modifiable risk factor for bacteraemia and death. Thomson P.C., Stirling C.M., Geddes C.C., Morris S.T., Mactier R.A. QJM 2007; 100: 415-422.

Gadolinium-Enhanced Magnetic Resonance Imaging and Nephrogenic Systemic Fibrosis – A Retrospective Study of a Renal Replacement Therapy Cohort. Collidge T.A., Thomson P.C., Mark P.B., Simpson K., Traynor J.P., Jardine A.G., Morris S.T., Roditi G.H. Radiology 2007; 245: 168-175.

Imaging peripheral disease: Gadolinium contrast may be risky in kidney disease [Letter]. Thomson P.C., Collidge T.A., Mark P.B., Traynor J.P. BMJ 2007; 334: 1335-1336.

A Prospective Observational Study of Catheter-related Bacteraemia and Thrombosis in a Haemodialysis Cohort: Univariate and Multivariate Analyses of Risk Association. Thomson P.C., Stirling C., Traynor J.P., Morris S.T., Mactier R.A. Nephrol Dial Transplant 2009 (in press).

List of Abbreviations

(G)	Catheter Exchange over Guidewire
A/CRF	Acute on Chronic Renal Failure
ACR	American College Radiology
APTT	Activated Partial Thromboplastin Time
ARF	Acute Renal Failure
AVF	Arteriovenous Fistula
AVG	Arteriovenous Graft
BHI	Brain Heart Infusion
BMI	Body Mass Index
bMIC	Minimum Inhibitory Concentration in Biofilm
CAPD	Continuous Ambulatory Peritoneal Dialysis
CE-MRV	Contrast-Enhanced Magnetic Resonance Venography
CFU	Colony Forming Units
CI	Confidence Interval
CKD	Chronic Kidney Disease
CRB	Catheter-Related Bacteraemia
CRF	Chronic Renal Failure
CRP	C-Reactive Protein
CVC	Central Venous Catheter
DBP	Diastolic Blood Pressure
ECP	Extracorporeal Photophoresis
eGFR	Estimated Glomerular Filtration Rate

EPR	Electronic Patient Record
ERF	Established Renal Failure
FDA	US Food and Drug Administration
Fem	Femoral Vein Insertion Site
HDL	High-Density Lipoprotein
HR	Hazard Ratio
IJug	Internal Jugular Vein Insertion Site
MH	Mueller-Hinton
MIC	Minimum Inhibitory Concentration
MRI	Magnetic Resonance Imaging
MRSA	Methicillin Resistant Staphylococcus Aureus
MSSA	Methicillin Sensitive Staphylococcus Aureus
NCTC	National Collection of Type Cultures
NKF-KDOQI	National Kidney Foundation Kidney Disease Outcomes Quality Initiative
NSF	Nephrogenic Systemic Fibrosis
NTCVC	Non-Tunnelled Central Venous Catheter
OD	Optical Density
PFGE	Pulsed-Field Gel Electrophoresis
pMIC	Minimum Inhibitory Concentration in Planktonic Solution
PTFE	Polytetrafluoroethylene
RRT	Renal Replacement Therapy
SBP	Systolic Blood Pressure
SD	Standard Deviation

SMRSARL	Scottish MRSA Reference Laboratory
SRR	Scottish Renal Registry
TCVC	Tunnelled Central Venous Catheter
UKRR	United Kingdom Renal Registry
USRDS	United States Renal Data System

Summary

The aim of this thesis was to perform a detailed evaluation of the risks to health conferred by haemodialysis vascular access and its maintenance.

The first part of this thesis examines the relationship between haemodialysis vascular access, bacteraemia and mortality. A retrospective analysis of vascular access type and subsequent outcome in all 265 prevalent haemodialysis patients attending Glasgow Royal Infirmary Renal Unit on a given date was conducted. Multivariate analyses of clinical and laboratory variables demonstrated an especially strong, independent relationship between the type of haemodialysis vascular access in use and risk of bacteraemia and death. When considering bacteraemia tunnelled catheters and non-tunnelled catheters were each independently associated with adverse outcome compared to arteriovenous fistulae (tunnelled HR 5.43 ($p<0.001$); non-tunnelled HR 3.14 ($p=0.01$)). When considering mortality tunnelled catheters and non-tunnelled catheters were each independently associated with adverse outcome compared to arteriovenous fistulae (tunnelled HR 2.75 ($p=0.012$); non-tunnelled HR 3.39 ($p=0.001$)).

The role of the central venous catheter in determining outcome was then explored in greater detail by a two-year prospective analysis of all patients undergoing haemodialysis catheter insertion in Glasgow Royal Infirmary. This study evaluated independent risk association between clinical and laboratory variables and instances of catheter-related bacteraemia and catheter thrombosis for each catheter type by univariate and multivariate analysis. Over the period of observation 823 central

venous catheter insertion procedures were conducted. Tunnelled central venous catheters (TCVCs) were associated with lower rates of bacteraemia and thrombosis than non-tunnelled central venous catheters (NTCVCs), independent of whether patients have acute or chronic renal failure, high levels of comorbidity and levels of routinely recorded biochemical and haematological parameters. Using TCVCs as a comparator, a hierarchy of risk association within the NTCVC insertion procedures was found with catheterisation of the internal jugular veins to be the best NTCVC insertion procedure (bacteraemia HR = 2.93, $p < 0.001$ / thrombosis HR = 4.65, $p < 0.001$), followed by insertion of a femoral venous catheter (bacteraemia HR 5.97, $p < 0.001$ / thrombosis HR = 9.23, $p < 0.001$). Catheter-exchange over a guidewire was associated with especially high risks of bacteraemia and thrombosis (femoral exchanges, bacteraemia HR = 9.84, $p = 0.002$ / thrombosis HR = 11.73, $p < 0.001$; internal jugular exchanges, bacteraemia HR = 6.42, $p < 0.001$ / thrombosis HR = 5.26, $p < 0.001$).

The second part of this thesis evaluates the performance of catheter lock solutions. This was explored by a prospective, randomised, controlled comparison of heparinised catheter lock solutions in 28 consecutive patients undergoing NTCVC insertion. Heparin 1000iu/mL incurred less disruption of systemic coagulation than heparin 5000iu/mL, without an obvious loss in clinical utility. When expressed as the percentage rise in activated partial thromboplastin time (APTT) at 10 minutes post catheter locking, the difference between groups amounted to a rise in APTT of 22.2% (range 0, 210) in the heparin 1000iu/mL group compared with 373.7% (range 133, 800) in the heparin 5000iu/mL group ($p < 0.001$).

An in-vitro assessment of combined anti-microbial/anticoagulant catheter lock solutions was then conducted. This studied six isolates of *staphylococcus aureus* in planktonic and biofilm models with regard to their response when exposed to heparin and vancomycin solutions alone and in combination. Heparin had the capacity to enhance and suppress staphylococcal growth, depending upon the strain studied. When combined with vancomycin heparin appeared to increase its potency although not in a dose-dependent way. The relative resilience of biofilm-embedded staphylococci to vancomycin compared with planktonic staphylococci was demonstrated.

The third part of the thesis begins with a retrospective evaluation of 78 consecutive magnetic resonance venograms conducted in haemodialysis patients with poorly functioning vascular access. A significant association was found between the frequency of vascular access procedures and the frequency of acquired structural abnormalities of the central venous system seen on magnetic resonance venography (central venous stenosis on imaging; arteriovenous fistulae or grafts $p=0.04$, central venous catheters $p=0.034$). It was noted that within this cohort a significant proportion of patients were found to have developed the relatively new disease nephrogenic systemic fibrosis (NSF).

The next chapter describes a case-control analysis of NSF cases in all 1826 patients who underwent renal replacement therapy over a six and a half year period in North Glasgow. This demonstrated a significant association between gadolinium-enhanced magnetic resonance imaging (MRI) and development of NSF (proportion of patients exposed to MRI, non-NSF 408/1812, 22.5% v NSF 13/14, 92.9%, $p<0.001$). Patients

with NSF were exposed to a significantly higher total dose of gadodiamide than their non-NSF counterparts (median 45ml v 30ml, $p<0.001$), indicating dose-dependency. This was further supported by weight-adjusted data (median 0.39mmol/kg v 0.23mmol/kg, $p=0.008$).

In conclusion, the work described in this thesis demonstrates the relative strength and independence of association between haemodialysis vascular access type and risk of mortality, bacteraemia and catheter thrombosis. Greater clarity has been demonstrated on the relative effect of heparin-based haemodialysis catheter lock solutions on markers of systemic coagulation in vivo, whilst the in-vitro variability of antimicrobial activity against planktonic and biofilm-embedded staphylococci with heparin and vancomycin, alone and in combination, has demonstrated the need for more rigorous evaluation and selection of potential haemodialysis catheter lock solutions. New insights were gained into the risks and benefits of contrast magnetic resonance venography in the assessment of vascular access in haemodialysis patients.

Chapter 1

Introduction

1.1 Chronic Kidney Disease

Chronic kidney disease (CKD) is an important condition with considerable public health implications. It affects a significant proportion of the general population and, when progressive, has an increasing impact on morbidity and mortality. CKD is an independent risk factor for cardiovascular disease and the development of established renal failure (ERF). Once a patient reaches ERF, quality of life has become invariably poor and life expectancy considerably shortened. Through the provision of renal replacement therapy (RRT) survival and quality of life of ERF patients can be markedly improved.

CKD places a considerable demand on medical resources around the world. The United States Renal Data System (USRDS) report of 2008 describes expenditures associated with CKD as having more than doubled over the past 10 years, from 11.3% of the total Medicare budget in 1996 to 24.5% in 2006. Costs for Medicare patients with CKD exceeded \$49 billion in 2006. The increasing cost of CKD to the US population is demonstrated in figure 1.1.

When considering the cost of providing RRT to the ERF population, the cost per patient rises significantly. In 2006 the prevalence of ERF patients maintained on RRT in the United States was 506,256. In that year total Medicare spending on patients with ERF rose to \$23 billion which accounted for 6.4% of the entire Medicare budget (1).

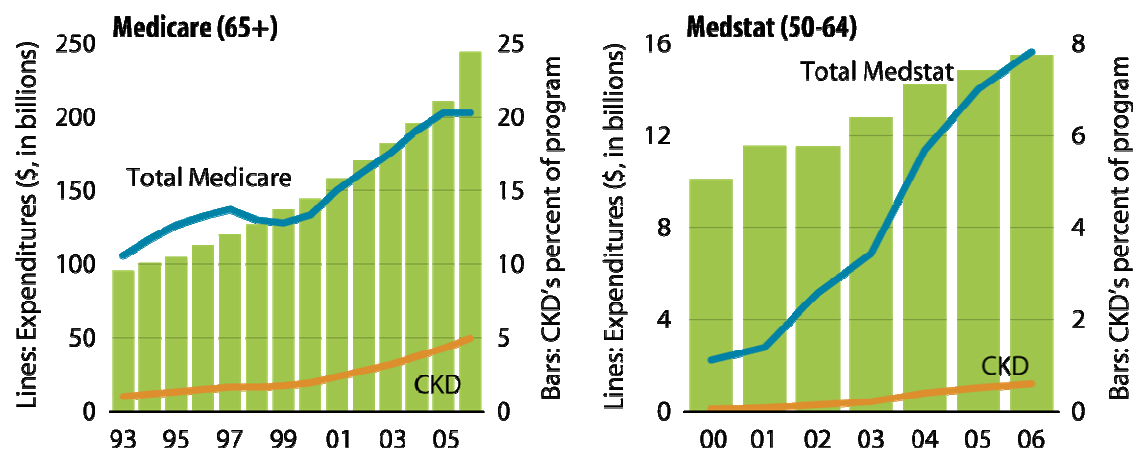


Figure 1.1 - Expenditure in the Medicare (1993-2006) and Medstat (2000-2006) populations of the United States. (Adapted, with permission, from the USRDS Annual Report, 2008).

In order to understand the resource implications of CKD and ERF, there is a pressing need to develop our understanding of their epidemiology. Historically, many estimates of CKD incidence were drawn from population studies using varied, arbitrary definitions of renal impairment. In one such study in 2003, by defining CKD as a serum creatinine level of $\geq 150\mu\text{mol/L}$ for 6 months, the annual incidence of detected CKD was determined as 1,701 per million population (2). An earlier study of advanced renal failure used a cut off of $>500\mu\text{mol/L}$ and reported an incidence of 148 patients per million population (3). Whilst these data were of significant interest, their clinical applicability was undermined by the use of serum creatinine as opposed to glomerular filtration rate when wishing to consider the absolute level of renal function.

The publication of the NKF/DOQI clinical guidelines in 2002 greatly improved our ability to study the epidemiology of CKD. These guidelines gave a universally applicable definition of CKD as either kidney damage for ≥ 3 months accompanied by

pathological or functional abnormalities of the kidney or a glomerular filtration rate of $\leq 60 \text{ mL/min/1.73m}^2$ for ≥ 3 months. This definition was further refined by subdivision into a 5-stage CKD classification. Stage 1 was defined as an estimated glomerular filtration rate (eGFR) of $>90 \text{ mL/min}$ and either $>20 \text{ mg/dL}$ of albumin in the urine, an albumin/creatinine ratio $\geq 30 \text{ mg/g}$, or other evidence of structural kidney damage. A similar definition was applied to stage 2 although with an eGFR range of 60–89. Stages 3, 4, and 5 were defined solely by eGFR ranges of 30–59, 15–29, and less than 15, respectively (4). By applying this structure to US data from the National Health and Nutrition Examination Survey (NHANES), the prevalence of CKD stages 1, 2, 3 and 4/5 was determined as 3.2%, 4.1%, 7.8% and 0.5% respectively. Recent USRDS data from 1999-2004 suggest an overall CKD prevalence of 16.8% in the U.S. population aged ≥ 20 years (5). This relatively high prevalence translates into a significant lifetime risk of developing ERF. In the US in 2002, Kiberd and Clase determined cumulative lifetime risk of ERF for a 20-yr-old black female, black male, white male and white female as 7.8%, 7.3%, 2.5%, and 1.8% respectively. These risks equated to the lifetime risks of breast, prostate and colorectal malignancies in the same population (6).

There is a relative wealth of data on the epidemiology of ERF compared with that of CKD. This is due to many countries with established RRT services engaging in comprehensive data collection programs overseen by national registries. These data have proven an invaluable resource in assessing service provision, establishing clinical standards and providing reproducible audit measures. Such data, collected year on year, have provided an insight into recent and expected trends within the RRT population.

1.2 Current Trends in Renal Replacement Therapy

The provision of renal replacement therapy (RRT) prolongs survival in patients with established renal failure (ERF) and in most cases improves quality of life. RRT may be provided by renal transplantation, haemodialysis or peritoneal dialysis. Over recent years the prevalent number of patients on RRT in the UK and around the world has been rising (figure 1.2) (7).

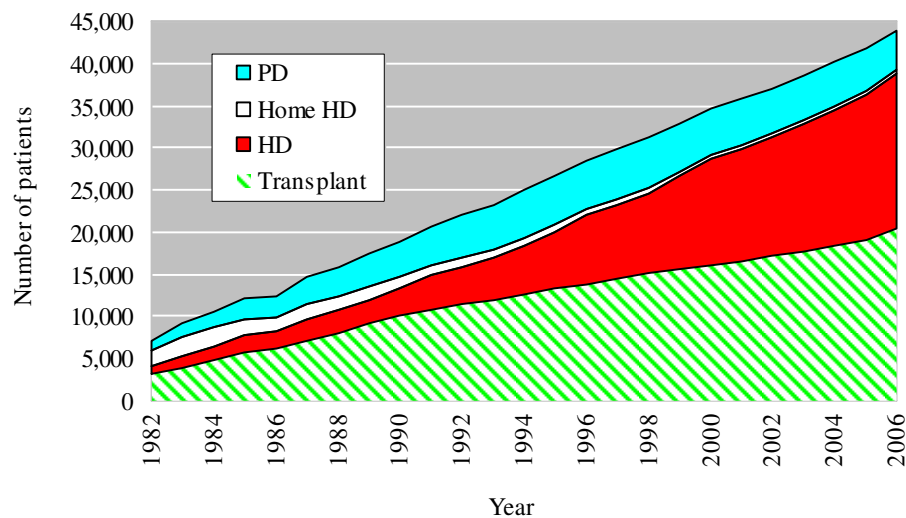


Figure 1.2 – Growth in prevalent UK patients by treatment modality at the end of each year 1982-2006 (Adapted, with permission, from the UK Renal Registry Tenth Annual Report, 2007).

At the end of 2006, the UK Renal Registry reported the prevalence of adult patients receiving renal replacement therapy in the UK as 43,901. This amounts to a UK prevalence of 725 per million population (7). Over recent decades the UK population prevalence of RRT has risen steadily in line with other developed countries within Europe, Australasia and the Americas (1, 8, 9). This is predominately due to a trend of rising incidence of starting RRT over recent decades (figure 1.3) and slowly increasing survival (figure 1.4) (10).

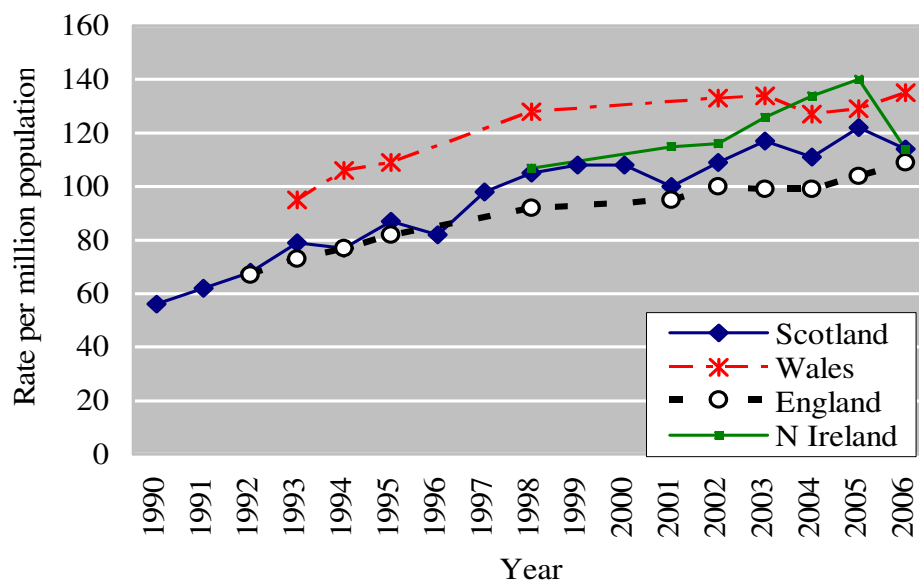


Figure 1.3 - Incident rates of RRT uptake in the countries of the UK: 1990-2006. (Adapted, with permission, from the UK Renal Registry Tenth Annual Report, 2007).

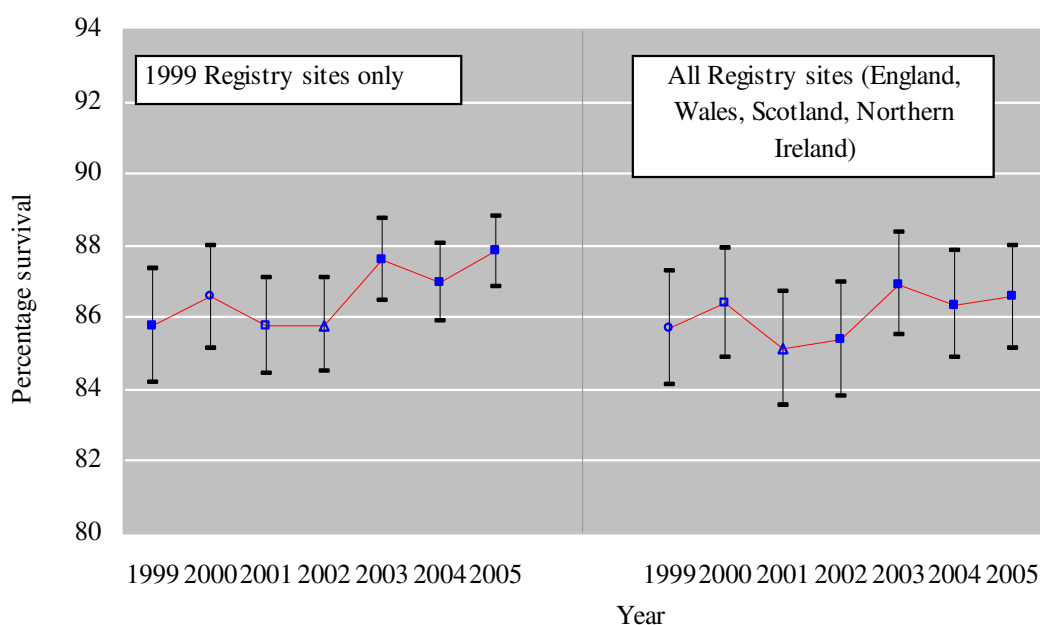


Figure 1.4 - Change in one-year, after 90 day, adjusted (age 60) survival, 1999-2005 within the UK Renal Registry participating centres from 1999 only (left) and all centres over time within the UK (right). (Adapted, with permission, from the UK Renal Registry Tenth Annual Report, 2007).

In 2006 the incidence of starting RRT in the UK was 113 per million population, a rise from 110 per million population in 2005. The age adjusted (60 years) one-year survival for the prevalent RRT population was 87.7% in 2006 compared with 84.9% in 2000 (7). The rise in RRT incidence over recent decades is predominately due to an increase in the availability of renal replacement therapy programs around the world. This has led to more inclusive RRT acceptance policies with a resultant expansion in the numbers of elderly patients and those with comorbidity such as diabetes and vascular disease. This phenomenon is demonstrated in figure 1.5 below.

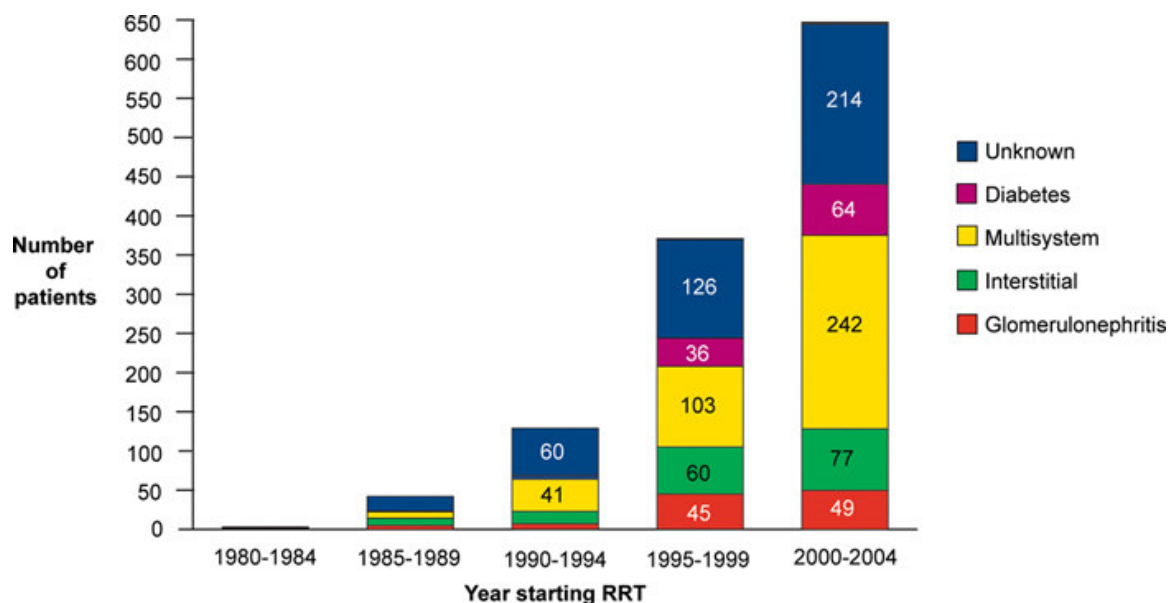


Figure 1.5 – Primary renal diagnosis of patients aged 75 years and older starting RRT in Scotland 1980-2004. (Adapted, with permission, from the Scottish Renal Registry Report 2002-2004).

The elderly now represent the fastest growing group of prevalent patients on RRT. An analysis of age ranges within the Scottish RRT population over the past 50 years is demonstrated in figure 1.6. This shows a steady expansion in the proportion of patients aged between 65 and 74 years of age, and those greater than 75 years of age. In 2004 the median age of patients starting RRT in Scotland was 65.3 years compared

with a median age of 60.9 years in 1995 (11). These findings are similarly reflected in data recently reported by the UK Renal Registry, European Renal Registry and USRDS (1, 7, 9).

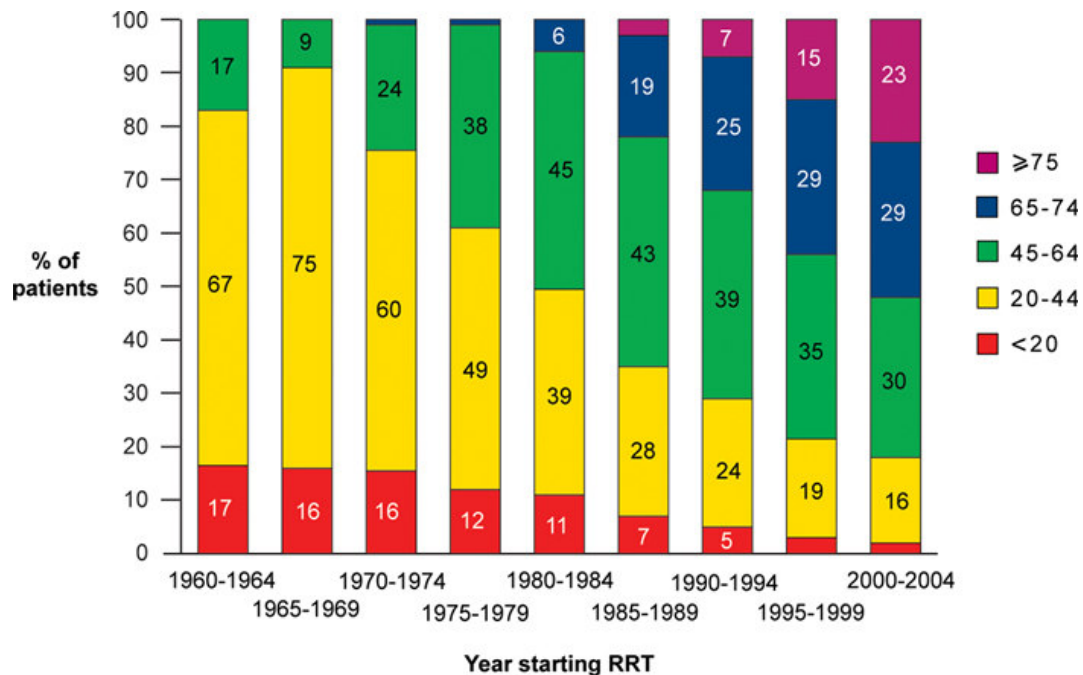


Figure 1.6 – Age distribution of patients when starting RRT 1960-2004. (Adapted, with permission, from the Scottish Renal Registry Report 2002-2004).

It also appears that as the RRT population ages, the burden of comorbidity within the population increases. The UK Renal Registry publishes a standardised list of accepted comorbid conditions. This contains a range of diagnoses covering cardiovascular diseases, diabetes, chronic obstructive pulmonary disease, liver disease, malignancy and smoking. From this list, the degree of comorbidity expressed within the UK RRT population has been found to vary between ethnic groups, with white patients tending to exhibit a higher prevalence of comorbid illness than those patients from Black, Asian, Chinese or other ethnic populations. Throughout all ethnicities, however, the burden of comorbidity has been shown to steadily increase

with age. This is demonstrated in figure 1.7 which displays the proportion of patients with a comorbid illness within differing age ranges. These data suggest that over two-thirds of the RRT population over 65 years of age will have at least one comorbid illness compared with one-third of the RRT population less than 44 years of age.

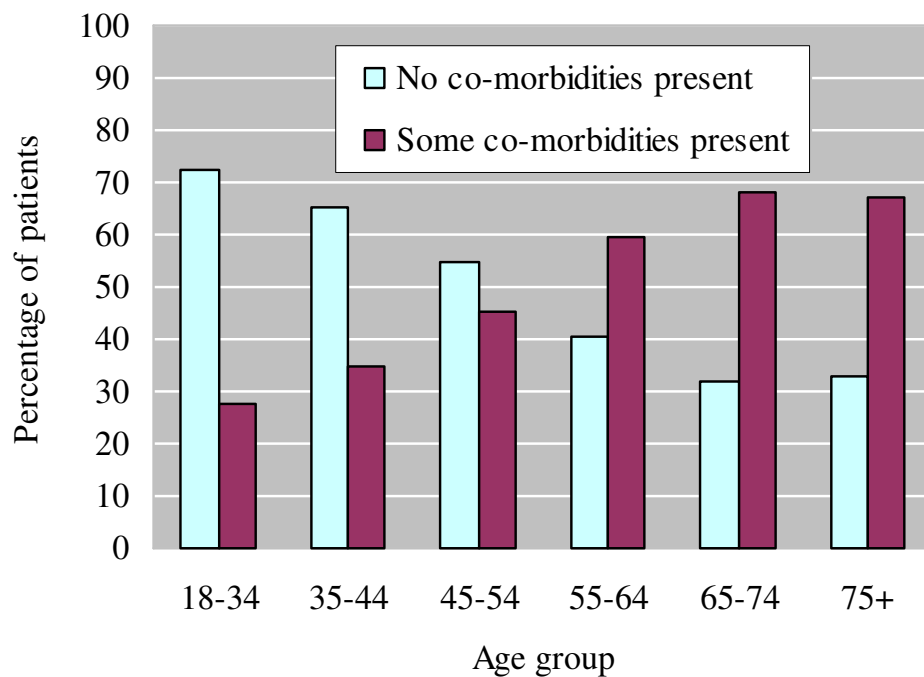


Figure 1.7 - Presence or absence of co-morbid conditions at the start of RRT amongst patients of White origin starting RRT (2001-2006). (Adapted, with permission, from the UK Renal Registry Tenth Annual Report, 2007).

The implications of an aging, more comorbid RRT population are significant. Data from the Scottish Renal Registry supports the association between age and comorbidity with adverse clinical outcome (12, 13). Whilst overall survival on RRT has increased, considerable variability in survival rates may be seen within the RRT population when considering the different age groups and the different primary renal diagnosis groups contained within. Data from the Scottish Renal Registry describe a median survival of 27.5 yrs in those patients under 20 years of age, falling to a median

survival of 1.3 years in those over 75 years of age. When considering primary renal diagnosis, patients with interstitial renal disease or glomerulonephritis may be expected to have a median survival of 9.3 years compared with 2.2 years in those with a multisystem disease (11). Consequently, as the age and proportions of constituent primary renal disease within the RRT population evolve, it can be anticipated that expected survival will change accordingly.

The dramatic difference in survival between the RRT population and the general population is illustrated below in figure 1.8. This figure demonstrates the considerable burden of cardiovascular disease within the ERF population and how it translates into heightened rates of mortality (14).

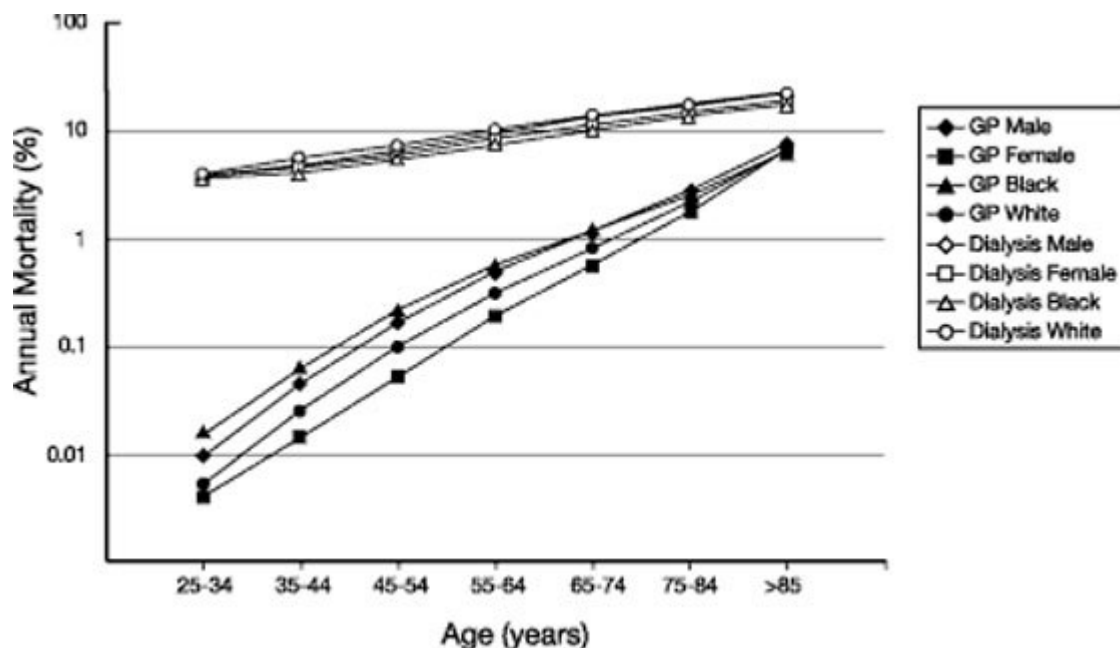


Figure 1.8 - Cardiovascular mortality in the US general population (GP) and in ERF treated by dialysis. (Recreated with permission).

This pattern has been elicited in several other studies of RRT populations in the developed world (8, 15). These findings not only reflect the high prevalence of traditional cardiovascular risk factors within the ERF population such as hypertension, left ventricular hypertrophy (16, 17) diabetes and proteinuria, but also the presence of risk factors generally specific to ERF such as vascular endothelial dysfunction (18-20), the development of atherogenic lipid profiles (21, 22) and vascular calcification (23). Many of these acquired risk factors have been found to develop early in the development in chronic kidney disease, and are associated with escalating cardiovascular risk as ERF approaches.

As the demographics of the RRT population change, dependence on the different modes of RRT provision also appears to be changing. When considering the prevalent UK RRT population, on the 31st December 2006, 44.9% were maintained on a functioning transplant, 44.3% maintained on haemodialysis and 10.6% maintained on peritoneal dialysis. Whilst the proportion of patients maintained on a functioning renal transplant appears to have been stable over recent years, the proportion of patients on haemodialysis appears to be increasing with a concurrent decrease in the proportion maintained on peritoneal dialysis. This trend may be partly explained by the increasing age and comorbidity of the RRT population limiting patients' ability to meet the physical demands that peritoneal dialysis incur.

Registry data have demonstrated an increasing trend in the overall frequency of haemodialysis as the first modality of RRT. In the UK in 2006, haemodialysis was the first modality of RRT in 76.6% of ERF patients compared with 58% in 1998. Of

the remainder 20.0% started RRT on peritoneal dialysis and 3.4% with a pre-emptive renal transplant. Increasing age is also associated with a greater likelihood of starting RRT on haemodialysis. When considering incident dialysis patients 90 days after starting RRT, 83% of patients over 65 years of age were started on haemodialysis compared with 70% of those less than 65 years of age (7).

In summary, the registry data accrued for the UK and other developed countries are suggestive of an RRT population that is increasing in size, age, comorbidity and its reliance on haemodialysis as the predominant means of delivering RRT.

1.3 Renal Replacement Therapy with Haemodialysis

RRT with haemodialysis does not provide true replacement of renal function. However by removing waste solutes, excess body water and restoring biochemical and acid-base balance, haemodialysis has considerably improved the morbidity and mortality of ERF patients.

The first description of dialysis was made by Thomas Graham of Glasgow in 1854. In his pursuit of the study of colloids, Graham was able to demonstrate a method of separating a mixture of colloids and crystalloids using a “dialyser”, a wooden hoop covered on one end by a selectively permeable membrane which floated on the surface of a mixed solution of crystalloid and colloid (24).

By applying Graham's principles Abel, Rountree and Turner developed the first artificial kidney in 1913 when they published on the removal of diffusible substances from circulating blood in living animals by dialysis. This was conducted in dogs and rabbits, and described the first successful in-vivo haemodialysis (25). The first occasion haemodialysis was performed in a human was by Haas in 1924, however it was another 20 years before Willem Kolff overcame the difficulties of providing adequate vascular access, establishing safe and effective anticoagulation and producing reliable equipment for widespread use when he created the rotating drum kidney in 1943 (26).

Kolff's original design was further refined in the early 1950s with the emergence of the stainless steel Kolff-Brigham dialysis machine. This was used for the treatment of acute renal failure as sustainable vascular access for repeated dialysis treatments was still difficult to obtain and effectively prevented its use as a long-term treatment for ERF. Further refinement of the Kolff dialysis machine and improvement in methods of establishing vascular access were led by Nils Alwall in Sweden and Belding Scribner in the US. Their work led to increasing numbers of patients with acute renal failure being treated by haemodialysis from the mid 1940s to the 1960s. By then many other centres were improving both the design of the haemodialysis machine and methods of establishing sustainable vascular access. In 1962 the world's first outpatient dialysis facility was opened in Seattle by Scribner and from then on demand for provision of RRT by haemodialysis facilities spread around the world.

The current haemodialysis machine bears little resemblance to that devised by Kolff in 1943 although the design adheres to similar principles. These centre on removing

blood from the intravascular compartment, passing it through an extracorporeal circuit into a dialyser and removing waste solutes and excess water by exposure to conditions that promote diffusion, convection and movement in response to hydrostatic pressure gradients. Dialysed blood is then returned to the patient via the venous system.

The volume of plasma cleared of solute per unit time by dialysis is expressed as the solute clearance. Diffusion is the predominant method by which solute clearance from plasma is achieved by haemodialysis. The process of diffusion is dependent upon blood from the extracorporeal circuit flowing through the dialyser, a collection of microfilament fibres bathed in dialysate fluid which circulates in the opposing direction to blood flow. These conditions are favourable to the rapid diffusion of solutes through pores within the microfilament fibres, down a concentration gradient from blood to dialysate or vice-versa. The rate of diffusion varies with the degree of concentration gradient between compartments, the surface area of the microfilament membrane, the number and size of pores within the membrane, the molecular size of the solute and the relative flow rates of both extracorporeal blood and dialysate. These factors determine the capacity of a dialyser and are collectively described as the mass transfer coefficient (K_oA) of the dialyser, a quantitative measure of the dialyser's efficiency of clearance.

Convection describes the movement of fluid between blood and dialysate compartments where, with increasing volumes of fluid movement from extracorporeal to dialysate circuits some solute may be concurrently dragged across the membrane and thus may contribute to the overall solute clearance achieved. Such movement is a key contributor in expressing the degree of flux exhibited by the microfilament

membrane in the dialyser. The concept of flux has arisen from a greater understanding of the clearance of small, medium and large molecular weight molecules. High-flux membranes have characteristically high permeability for water, low and middle molecular weight solutes and most are made from materials of high biocompatibility. While transport of solutes through low-flux membranes is mainly achieved by diffusion, convection plays an especially important role in the performance of high-flux membranes.

Fluid is predominately removed by the production of a transmembrane pressure gradient across the microfilament membrane. This causes fluid to cross from the relatively high pressure within the extracorporeal circuit to the relatively low pressure created on the dialysate side of the membrane. This process of fluid removal from the extracorporeal circuit is termed ultrafiltration. The degree of ultrafiltration achieved across a membrane is a function of its permeability, surface area and the size of the transmembrane pressure gradient and may be expressed as the ultrafiltration coefficient.

It can be seen that a key determinant in the success of haemodialysis are the characteristics of the selectively permeable membranes used to form the microfilament tubes within dialyser units. Membranes may now be classified by the type of material used in their manufacture (synthetic, cellulose, substituted cellulose), their capacity, surface area, ultrafiltration coefficient, flux and, in some cases, their ability to be reused.

The prescription of haemodialysis therefore requires consideration of its constituent components. Alteration of the KoA, ultrafiltration coefficient and flux all have the ability to affect the amount of dialysis delivered and the relative clearances of small, medium and large molecular solutes. Despite greater understanding of haemodialysis method and significant advances in haemodialysis technology, the morbidity and mortality of dialysis patients has remained disproportionately high. Whilst it has been demonstrated that low doses of delivered haemodialysis are associated with adverse outcome (27) the precise components of the optimal haemodialysis prescription remain controversial and have yet to be fully determined by randomised controlled trials (28-30).

One component, however, has consistently been shown to be fundamental to the effective delivery of haemodialysis – the successful provision of functioning vascular access. Without effective and sustainable methods of establishing and maintaining vascular access, haemodialysis, irrespective of modality, cannot be provided.

1.4 Methods of Haemodialysis Vascular Access

Successful haemodialysis depends on the provision of safe, efficient and durable vascular access. Establishing and maintaining effective vascular access is a demanding process for both patients and renal services. These demands are set to increase in response to an RRT population that is becoming increasingly reliant on haemodialysis whilst also increasing in size, age and comorbidity.

Initially, vascular access methods relied on repeated peripheral cannulation to deliver arterial blood to the dialysis machine and return it to an accompanying vein. In 1949, Alwall from Lund, Sweden, made the first attempt to make a direct connection between an artery and a vein, using glass cannulae and rubber tubing (26). This device would allow blood to be diverted onto an extracorporeal circuit for dialysis when required. His attempt was unsuccessful although it provided the template for the arteriovenous Teflon Shunt developed by Quinton, Dillard and Scribner in the US in 1960 (31). Their device consisted of two Teflon cannulae inserted into the wrist, one in the radial artery and one in accompanying cephalic vein. The external ends of the cannulae were connected by flexible tubing from which connection to an extracorporeal circuit could be made. This provided nephrologists with the first permanent vascular access device and was a decisive breakthrough in the provision of haemodialysis to the ERF population. The 'Scribner' shunt, as it became known, underwent many refinements before being ultimately superseded by the successful development of arteriovenous fistulae, arteriovenous grafts and central venous catheters. Nonetheless, the Scribner shunt played a key role in the development of permanent vascular access devices.

The Arteriovenous Fistula

In 1962, Cimino and Brescia described a technique where haemodialysis was conducted through a simple puncture of the most accessible forearm vein. Patency of the vein was assured by the use of an inflatable tourniquet. This allowed needles of varying sizes to be used with resultant haemodialysis flows of 150-400mL/min. Whilst successful, this technique was limited by the poor longevity of peripheral veins

in comparison with that of Scribner's external arteriovenous shunt. Logical development of this technique, however, led to the creation of the first internal arteriovenous fistula (AVF). The successful use of the new technique was reported in a landmark paper published by Brescia et al in 1966. They reported 12 cases in which successful primary function of an AVF had been achieved by creating a side-to-side anastomosis between the radial artery and the cephalic vein at the wrist. Exposure to high-pressure arterial flow was found to promote enlargement and thickening of the venous wall. After approximately six weeks maturation a robust vessel wall had developed that could sustain repeated cannulation and allow regular haemodialysis to take place (32).

A year later the technique had been amended to allow successful creation of an end-to-end anastomosis between radial artery and cephalic antebrachial vein in the forearm (33). This technique restricted arterial inflow into the AVF to that delivered by the feeding radial artery and thus led to a high risk of developing steal syndrome. Consequently the technique latterly became regarded as a secondary option available to surgeons when considering surgical revision of a failed AVF.

The technique was further refined in 1968 by Rohl, who devised the radial artery-side-to-vein-end anastomosis, with or without ligation of the radial artery distal to the anastomosis. This allowed a more suitable positioning of the AVF with excellent subsequent flows. It is this technique that has become the standard AVF creation procedure of choice and has allowed AVF creation surgery to evolve and successfully provide a range of potential sites for AVF creation, predominately within the upper

limbs (26). A diagrammatic representation of an upper limb AVF is demonstrated in figure 1.9.

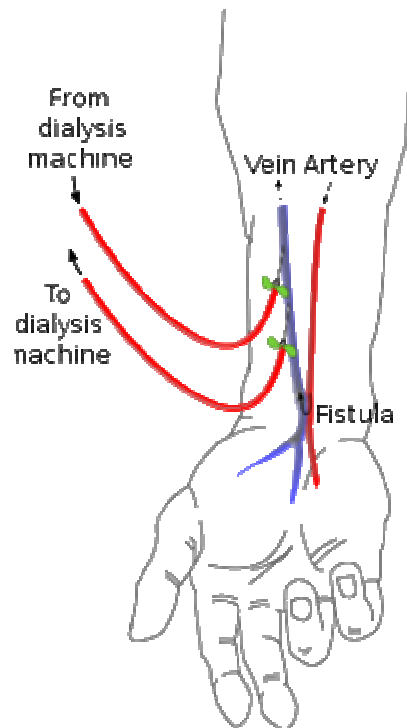


Figure 1.9 – A typical radiocephalic arteriovenous fistula with cannulation points for attachment to the extracorporeal haemodialysis circuit.

For practical purposes AVF creation is best conducted on the non-dominant arm with use of distal sites where possible; preserving the proximal vascular tree should vascular access surgery be required in the future. The longevity, durability and favourable complication rate of the AVF have established it as the leading method of establishing permanent haemodialysis vascular access. Approximately 68% of haemodialysis patients in the UK dialyse via an AVF at present (34).

Synthetic Arteriovenous Grafts

An alternative to the AVF is the synthetic arteriovenous graft (AVG). This was devised following the introduction of the Scribner shunt, which was noted to employ a length of flexible tubing to connect the arterial and venous cannulae. In 1969 George Thomas developed this principle by replacing the cannulae with Dacron patches sutured into the vessel wall and bringing out a loop of connecting silastic material to the skin surface. By avoiding the use of intraluminal cannulae, this device was less prone to thrombosis (35). Meanwhile Buselmeier et al had adapted the Scribner shunt by using a silastic material which could be tunnelled subcutaneously to connect the two cannulae. During the same period the first vein graft had been performed using a length of excised saphenous vein to connect the brachial arterial to its accompanying vein. By combining each of these three principles – direct anastomosis of vessels to tubing, looping a section of tubing to connect artery to vein and subcutaneous tunnelling of the connecting loop, the modern AVG was created (26).

Whilst initially Dacron was the most commonly used graft material, the emergence of the synthetic material polytetrafluoroethylene (PTFE) as a pliable, biocompatible material that may be repeatedly cannulated yet maintain its structural integrity, led to significant improvement in the durability of the AVG. First described by Soyier in 1972 PTFE has since become the most commonly used graft material for haemodialysis vascular access worldwide (36). A diagrammatic representation of an upper limb AVG is demonstrated in figure 1.10

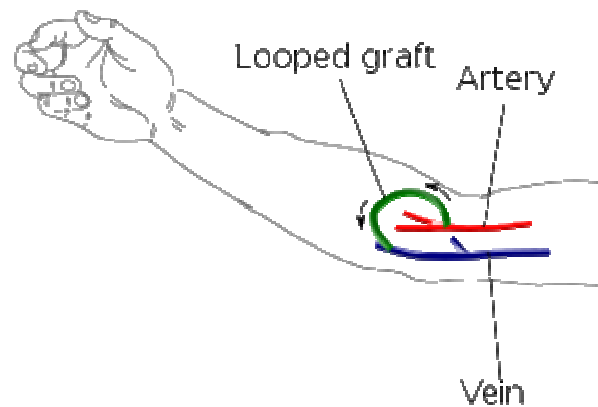


Figure 1.10 - A typical looped brachiocephalic arteriovenous graft used for attachment to the extracorporeal haemodialysis circuit.

Commonly an AVG is used to connect the brachial artery to the basilic vein, although grafts between the radial artery and basilic vein and the brachial artery and axillary vein are also regularly used. When vascular access in the upper limbs is exhausted, synthetic grafts can be used to establish vascular access using the subclavian or axillary vessels, femoral vessels, or even may be anastomosed between the arterial system and the right atrium. Approximately 3% of haemodialysis patients in the UK dialyse via an AVG at present (34).

Central Venous Catheters

In the early years of haemodialysis the demand for experienced surgeons to create arteriovenous shunts, fistulae and grafts outstripped supply. The paucity of vascular surgeons prepared to perform these procedures provoked one UK nephrologist, Stanley Shaldon, to develop hand-made cannulae that could undergo insertion into the femoral artery and accompanying vein to permit immediate haemodialysis access. He made use of the Seldinger insertion technique - a method that enables safe catheter

placement into the vascular tree introduced by Sven-Ivar Seldinger in 1953 (37). Arterial cannulation was soon abandoned after it was found to be associated with an unacceptably high risk of bleeding compared to veno-venous cannulation. Gradually different insertion sites were used including the jugular and subclavian veins. These had the advantage of allowing central venous pressures to be estimated in patients with extracellular fluid depletion (38), a common occurrence in many patients with acute renal failure requiring dialysis. Cannulation of the subclavian vein became the favoured approach for central venous catheter (CVC) insertion until the early 1990s when angiographic data demonstrated a significantly increased risk of central venous stenosis at the site of cannulation. This predisposed patients to a high risk of limb oedema which could impair the ability to create and maintain a functioning AVF (39). Insertion into the internal jugular veins is now regarded as standard practice although femoral venous cannulation is also performed.

CVCs are frequently used temporarily to provide vascular access for haemodialysis whilst the patient awaits creation or maturation of an AVF or AVG or because they have run out of suitable options for permanent vascular access. Some CVCs may be tunnelled subcutaneously en-route to entering the vein with a securing cuff to stabilise the position of the catheter and reduce periluminal infection. Direct transcutaneous cannulation of the vein is often performed acutely and tends not to involve subcutaneous tunnelling or use of a securing cuff. Polyurethane and silicone are the two materials most commonly used in the manufacture of haemodialysis catheters although polymers such as carbothane are increasingly common. These materials provide sufficient flexibility, durability and biocompatibility for intravascular use. Approximately 29% of haemodialysis patients in the UK dialyse via a CVC (34).

These different methods of obtaining vascular access allow haemodialysis to be a viable treatment for the majority of patients with ERF. The diversity of vascular access options available can help nephrologists address a range of clinical scenarios more effectively. Late presentation of ERF is one frequently experienced scenario that may have a significant impact on vascular access provision. In this setting the time in which RRT is required to start may arrive before the patient can undergo vascular assessment, surgery and successful maturation of their fistula or graft. This phenomenon is often used to explain the relatively high prevalence of ERF patients using CVCs as their first haemodialysis access modality. In the UKRR vascular access survey of 2006, 66% of patients started haemodialysis on a CVC compared with 34% using an AVF or AVG. By 12 months 28% of prevalent patients were using a CVC compared with 71% using an AVF or AVG (34). Similarly when an AVF or AVG fails, CVCs are a rapid means of establishing vascular access and thus have an important role in the emergency provision of vascular access.

Whilst each access type has its relative attributes, it is vitally important to consider the differing degrees of reliability, durability and complications associated with each approach. Whether considering an individual patient's circumstances or planning vascular access provision at a population level, understanding the range of complications expressed by each access type and which factors predispose to these complications is of fundamental importance in deriving maximum benefit with minimal risk.

1.5 The Complications of Haemodialysis Vascular Access

Each vascular access method has associated complications that may arise immediately following creation or later during regular use. Failure of vascular access is most frequently due to thrombosis occurring within or around the AVF, AVG or catheter however other significant problems such as infection, aneurysm formation, heart failure and ischaemia distal to the site of access creation may occur.

Establishing and maintaining vascular access in the face of these complications is costly. USRDS data suggest that the total annual cost of establishing and maintaining haemodialysis vascular access is approximately one billion dollars. The recent trend of increasing expenditure on dialysis access reported to the USRDS is demonstrated in figure 1.11 below.

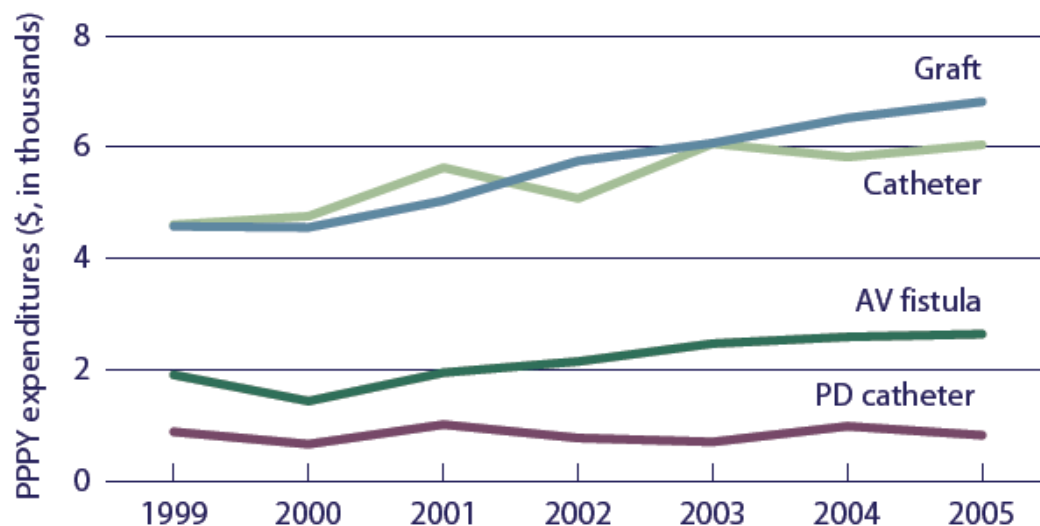


Figure 1.11 - Per person per year access costs, by type of access, in the USRDS ERF population (1999-2005). (Adapted, with permission, from the USRDS Annual Report, 2008).

Within these figures there has been a notable rise in the amount spent on interventional radiology services and a relative fall in the amount spent on vascular surgery and thus access creation costs have remained relatively static. The main rise in expenditure relates to vascular access maintenance with access-related complications accounting for nearly a quarter of hospitalisations in the RRT population (1).

Thrombosis

Over 80% of vascular access loss is due to local vascular thrombosis. In the case of arteriovenous fistulae or grafts, this is mostly due to intravascular stenosis from anatomical defects arising within venous drainage from the access site although rarely from the arterial inflow. The rate of AVF thrombosis is estimated as 0.2-0.4 episodes per patient year compared with 0.8-1.2 episodes per patient year for AVGs (40).

The main substrate for venous stenosis is endothelial cell injury, most commonly at the site of the anastomosis. This results in the up regulation of a pro-inflammatory cascade which promotes smooth muscle cell accumulation at the site of injury (41). This process may be escalated in response to shear stress arising from turbulent flow (42), repeated cannulation and the relative difference in vascular elasticity either side of the anastomosis (43). Other contributing factors include compression of the fistula or graft between dialysis sessions, hypotension, hypovolaemia and hypercoagulable states (44).

Primary prevention of AVF or AVG stenosis has focussed on selecting an appropriate site for vascular access creation, as determined by vascular imaging, and monitoring fistula or graft performance. Monitoring relies on assessment of access structure, assessment of access blood flow, measurement of delivered dialysis dose and assessment of the resistance to blood flow on return to the venous tree. These features may all contribute towards determining whether an AVF or AVG is at risk of failure. Where venous stenosis of >50% is discovered, percutaneous angioplasty or surgical revision may improve AVF or AVG longevity. Intervention on a thrombosed AVF, however, is not likely to lead to restoration of long-term patency unless an acute, unexpected deterioration in AVF performance occurs and patency is restored promptly. Monitoring may occur routinely as part of a regulated screening program or be undertaken in response to clinical deterioration in a previously functioning AVF or AVG. Best practice remains uncertain. Whilst many of the methods used in detecting venous stenosis and thrombosis are effective, routine screening with subsequent intervention has not yet been proven to reproducibly increase the long-term survival of AVFs and AVGs. Prospective monitoring thus remains controversial (45, 46). Some guideline groups are recommending some form of prospective monitoring for all AVFs and AVGs (47) whilst others recommend restricting detailed AVF and AVG assessment to those with clinical signs of deterioration such as a decrease in intradialytic blood flows (48).

The issue of antiplatelet agent use in preventing AVF or AVG thrombosis is unresolved. Several antiplatelet agents including aspirin, dipyridamole, clopidogrel, sulfinpyrazone and ticlopidine have been studied in the past. Many of the studies were conducted in the 1970s and 1980s where dialysis populations and treatments

were very different to those seen today (49). Recent studies have focussed primarily on primary patency in arteriovenous grafts. One more recent trial compared dipyridamole and/or aspirin in PTFE AVGs. Neither treatment approach appeared to be effective as a secondary preventative measure although primary prevention of thrombosis was demonstrated with dipyridamole (50). A randomised controlled trial evaluated use of aspirin and clopidogrel although was stopped early because of a significantly elevated risk of bleeding among those receiving antiplatelets (51). More recently a randomised controlled trial of 649 patients with a new AVG assessed dipyridamole (200 mg twice daily) plus aspirin (25 mg twice daily) or placebo. The intervention group demonstrated greater rates of primary patency with a similar rate of adverse events, including bleeding, albeit in a population designed to be of low bleeding risk and with relatively short exposure to the antiplatelets on trial (52). Use of anticoagulants such as warfarin has not been proven to reduce thrombosis and has been associated with an increased risk of bleeding (53).

When considering CVCs, thrombosis is a common problem that frequently results in catheter loss. Two types of thrombosis may occur with central venous catheterisation – extrinsic venous thrombosis and intrinsic catheter thrombosis. Extrinsic venous thrombus arises in lumen of the vein in which the catheter has been inserted, invariably around the outside of the catheter and thus impairing blood flow past the device. Whilst not necessarily attached to the catheter surface, the catheter or its tip may be embedded within the thrombus, further exacerbating abnormal venous flow patterns. Intrinsic thrombus arises from within the catheter lumen and may extend from the catheter tip outwards, reflecting back over the catheter as a surrounding sheath. Up to 70% of CVCs may develop a fibrin sheath soon after insertion.

Both extrinsic and intrinsic catheter thrombosis may arise from (i) abnormalities of blood flow such as turbulence and low venous flow rates; (ii) abnormalities of the vessel wall such as local vascular endothelial damage following catheter insertion and the presence of a foreign body within the venous lumen; and (iii) a local or systemic pro-coagulant state such as that mediated by the release of pro-inflammatory cytokines from the damaged venous endothelium or the presence of a systemic coagulopathy. Once local thrombosis occurs, venous flow is disturbed and an escalating cascade of thrombus formation may perpetuate.

Primary prevention of catheter thrombosis focuses on selection of an appropriate site for CVC insertion and adequate heparinisation of the catheter in between dialysis sessions. The internal jugular vein is favoured over the sub-clavian vein due to its lower risk of subsequent venous stenosis that could impair the function of future AVFs or AVGs. Many patients who have undergone previous vascular access procedures may benefit from imaging of target sites – a practice increasing in frequency with the development of interventional radiology services. Controversy exists as to the best means of assessing the central veins in complex patients, with ultrasound, radiocontrast venography and magnetic resonance venography all being available methods. Use of contrast enhanced magnetic resonance venography (CE-MRV) had increased over recent years due to its proven speed, accuracy and reproducibility in the detection and evaluation of central venous thrombus and stenosis (54). Recently, however, use of CE-MRV has come under close scrutiny following reports indicating an association between the administration of gadolinium-based contrast agents and the development of the rare condition nephrogenic systemic

fibrosis (NSF) (55). This has limited the use of CE-MRV in these patients and placed renewed focus on imaging practices in ERF patients.

The routine practice of filling the internal lumen of CVCs with heparin solution is almost universal in haemodialysis catheter use due to the clear benefit in preventing intraluminal thrombosis. The concentration and volume of heparin solution instilled into catheters may vary greatly between renal units. The benefits of maintaining catheter patency with heparin-locking are balanced against the risk of systemic heparinisation and subsequent bleeding. In a Canadian longitudinal study of 6940 haemodialysis sessions the use of a 1000iU/mL heparin lock solution in central venous haemodialysis catheters was not associated with higher rates of catheter malfunction compared with the use of 10,000iU/mL heparin solution but the 1000iu/ml group did require greater anti-thrombotic intervention with thrombolytic drugs (56). Concentrations of heparin up to 5,000iU/mL are regularly used yet no prospective studies have been performed to examine the effects of higher concentrations of heparin solution.

Several measures may be used to treat established catheter thrombosis. Catheter salvage may be attempted by infusion of saline flushes which are easily performed, economical, safe and frequently effective (57). Intraluminal thrombolytic or a systemic infusion of thrombolytic may also restore catheter patency. Historically this was conducted with urokinase however tissue plasminogen activator (tPA) has been demonstrated as providing better long-term patency without any additional risk of bleeding or other adverse events (58). Despite this, one prospective study found tPA infusion to allow for a median of only five to seven additional haemodialysis sessions

before needing repeated or the catheter needing exchanged. The overall clinical benefit and cost-effectiveness of tPA treatment thus remains doubtful (59).

Anticoagulation with warfarin as a means of preventing catheter thrombosis remains controversial. Sub-therapeutic warfarinisation has been found to be of no benefit to catheter patency in patients on haemodialysis (60). Warfarinisation to an INR range of 1.5 to 2.5 has been found to be of some benefit in patients deemed at high risk of thrombosis (61, 62). Recent USRDS data, however, have found the rate of development of subdural haematomas in long-term dialysis patients is 10 times higher than that of the general population and cite a concurrent increase in warfarin usage as being a possible contributant (63). Further data has also demonstrated an association between warfarin use and heightened risk of stroke in haemodialysis patients (64). Consensus opinion is therefore that anticoagulation with warfarin only be used in long-term haemodialysis patients when deemed absolutely necessary and when justified by published evidence.

Another option is catheter replacement over a guidewire with or without stripping of the fibrin sheath (65, 66). This approach allows optimal preservation of the venous anatomy with a significant rate of success. Doubts exist, however, when considering the likelihood of recurrent thrombosis and the prospect of introducing infection through the subcutaneous tunnel during catheter exchange. One small study of 42 catheter exchange procedures found comparable rates of subsequent infection and failure to de novo catheter insertions (67) however others have suggested an increase in complication rates (68). How the complication rate of catheter exchange

procedures compares with other catheter insertion procedures is unclear and remains an area of controversy.

Infection

Infection is a common problem in haemodialysis patients and accounts for increasing rates of hospitalisation, comorbidity and mortality. Figure 1.12 demonstrates recent trends in admissions for principle diagnoses of infection and bacteraemia/septicaemia in the USRDS ERF population. It has been found that approximately 20% of vascular access loss is due to the development of infection.

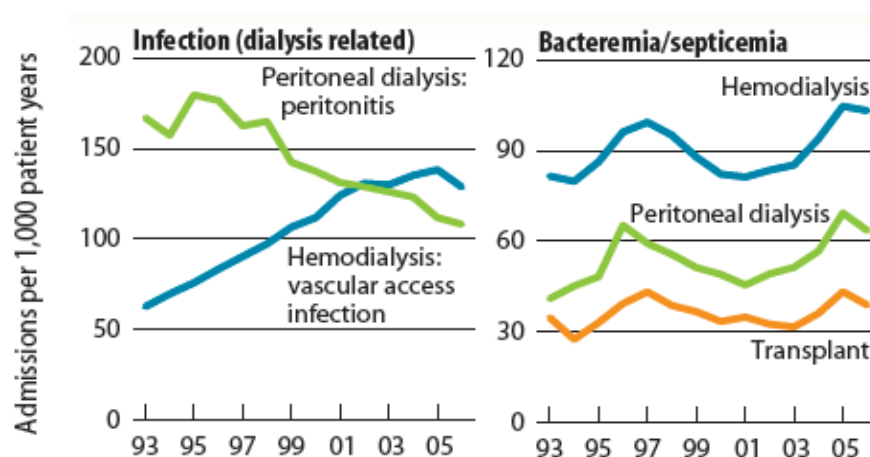


Figure 1.12 – USRDS 2008 report, adjusted admissions for principle diagnoses, by modality from 1993 to 2005. (Adapted, with permission, from the USRDS Annual Report, 2008).

Vascular access is the source of the majority of infections within haemodialysis units. The predominance of skin commensals as pathogenic organisms in bacteraemic patients suggests that inoculation during vascular cannulation is the main mechanism by which infection arises (69). Once inoculated, synthetic materials such as those

used in AVGs and CVCs have a tendency towards chronic infection due to their lack of innate immune defence mechanisms and their propensity to develop biofilm compared with autologous AVFs. Biofilm is a complex community of sessile cells that attach to a substratum and to each other. Bacteria may form biofilm through the production of a matrix of glycoprotein polymers that act as both a physical and chemical barrier to the immune response. These properties allow biofilms to develop resistance to biocides, phagocytes and antimicrobials with the degree of resistance correlating with the maturity of the biofilm (70). Biofilm formation is a key substrate in the development of chronic infection in vascular access devices. A scanning electron microscopic image of a staphylococcal biofilm is demonstrated below in figure 1.13.

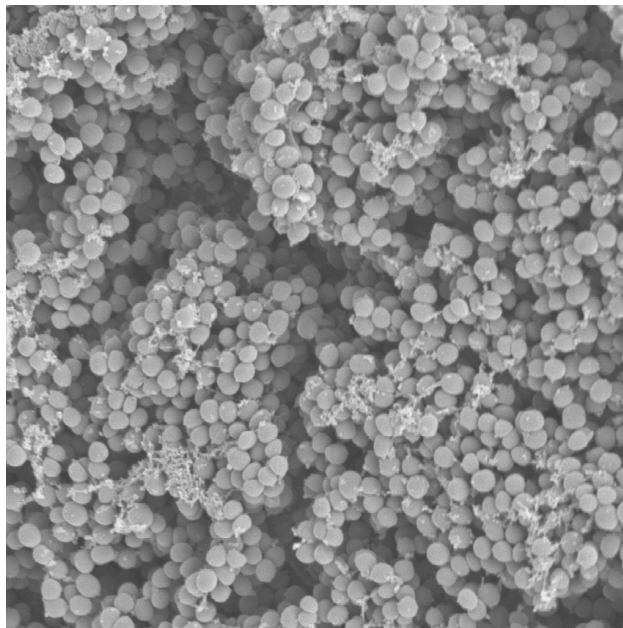


Figure 1.13 – A scanning electron microscopic image of a staphylococcal biofilm formed on a 96-peg lid plate.

Prevention and treatment of vascular access infection is a major component in the safe delivery of haemodialysis. When considering arteriovenous fistulae and grafts, sterile

cannulation is fundamentally important. Poor cannulation technique, often due to relative inexperience of dialysis personnel, may lead to pseudoaneurysm formation or the development of perifistular haematomas and is significantly associated with subsequent infection (71).

Routine antibiotic prophylaxis is controversial. Topical antibiotic use has not been found to be effective with benefits in reducing nasal staphylococcus aureus carriage by topical mupirocin treatment being found to be associated with the development of resistant pathogens (72). Consequently most primary prevention strategies focus on the ability to ensure aseptic technique when inserting or handling vascular access devices.

Practices such as sterile barrier nursing, hand hygiene, use of antiseptic solutions such as chlorhexidine and removal of the catheter at the earliest point possible are widely established and reflected in national guidelines (48).

When considering CVCs the location and type of catheter used have an important association with subsequent rates of infection. Infection is more commonly seen in patients who have undergone catheterisation of the femoral veins compared with those in whom catheterisation is undertaken in the internal jugular veins. Catheters tunnelled subcutaneously prior to entry into the vein are associated with lower rates of infection. Whether these associations arise due to the location and method of catheterisation in distinct sites or as a function of the characteristics of the types of patients who undergo different catheterisation procedures remains unclear.

New methods for the primary and secondary prevention of CVC infection have been devised over recent years with a growing evidence base detailing their applicability. Catheter materials coated or impregnated with anticoagulants, antibiotics, and silver ions have been studied with conflicting results. One meta-analysis of 11 randomised trials demonstrated significant reductions in catheter colonisation and catheter-related bacteraemia with antiseptic-impregnated catheters compared to standard non-impregnated catheters. These conclusions were disputed in a second meta-analysis which highlighted inconsistencies in key definitions, failures in determining clinically important end points and the neglect of potential confounding variables in many of the study populations examined (73). Despite these uncertainties such catheters are often found in routine use within dialysis centres.

Another area of increasing interest is the development of combined anticoagulant-antimicrobial catheter lock solutions. Known as the antibiotic lock technique, this method is targeted at preventing and eradicating biofilm on the endoluminal surface of haemodialysis catheters. An antibiotic-anticoagulant solution is instilled into the catheter lumen and left to dwell between haemodialysis sessions. This method delivers a small absolute amount of antibiotic to the patient but achieves a high local concentration in the catheter lumen that is 100 to 5,000 times higher than the minimum inhibitory concentration (MIC) for the infecting bacterium. Use of antibiotic lock solutions as a primary prevention strategy has raised concerns regarding the risks of developing antimicrobial resistance with leakage of the antibiotic-lock solution into the circulation. Using antibiotic-lock solution as a secondary adjunctive measure to systemic antibiotics in treating CRB is, however, less contentious.

A recent meta-analysis of the antibiotic lock technique was reported by Jaffer et al in 2008. This reported the relative success of antibiotic lock solutions in haemodialysis cohorts with a significant reduction in the frequency of catheter-related infection (74). A further meta-analysis using different inclusion criteria arrived at the same conclusions with the techniques appearing to offer significant improvements in bacteraemia rates (75). In both studies the authors, however, cited major limitations incurred due to the relatively short duration of follow-up and thus an inability to assess the risk of antibiotic resistance.

Other reported barriers to the use of these solutions are concerns of systemic leakage of the lock solutions, a finding demonstrated by one of the randomised controlled trials which used gentamicin 40mg/ml in combination with citrate where basal levels of gentamicin were detected in the systemic circulation (76). This may result in a cumulative increase in risk of toxicity/adverse effects. The potential economic costs of these solutions are also yet to be fully evaluated. Whilst this area continues to evolve, our understanding of how combinations of anticoagulant and antimicrobial interact is limited and remains an area of considerable interest.

1.6 Summary

It can be seen that when considering the complications of haemodialysis vascular access, many areas of controversy and uncertainty persist. This thesis consists of

several studies which aim to bring clarity and certainty to specific areas of vascular access practice and thus enhance clinical judgement and decision making.

The first part of this thesis scrutinises the hierarchy of complications seen across the range of haemodialysis access types. In particular the complication rates associated with different types of vascular access catheter insertion procedure are examined. The prevalence of maintenance procedures, thrombotic complications and infectious complications reported by each vascular access population in the USRDS 2008 survey are demonstrated in table 1.1 below.

Complication	AVF (%)	AVG (%)	CVC (%)
Removal	0.7	1.1	13.3
Replacement	0.7	1.3	25.4
Declotting	3.3	14.4	1.5
Mechanical Complication	0.5	0.8	1.6
Infection	1.1	2.6	10.2
Sepsis	4.2	4.8	15.7

Table 1.1 – A comparison of prevalent maintenance procedures, thrombotic complications and infectious complications reported by each vascular access population reported in the USRDS 2008 Data Report.

AVFs have the lowest complication rates followed by AVGs and then CVCs. Consensus opinion thus suggests that AVF creation should be sought where possible (47). Clearly there are logistical hurdles to this – late presentation to renal services, fitness for surgery, suitable peripheral vascular anatomy, delays due to primary or secondary access failure and slow rates of AVF maturation. Consequently there

remain situations, especially when starting RRT, where use of an AVG or CVC may be required. This is demonstrated by the relative preponderance of CVC use in patients starting RRT around the world. Similarly CVC insertion is the mainstay of vascular access provision to the acute renal failure population who require haemodialysis. CVCs therefore have an important role in haemodialysis vascular access provision although do so at a cost.

USRDS data from 2007 suggest that expenditure on catheter placement is approximately 2.5 to 2.8 times higher than on AVF and AVG surgery. It is suggested that these figures reflect the excess costs the catheter group incur by being a predominately in-patient comorbid population compared to the population who undergo elective AVF and AVG surgery (1).

The association between catheter use, comorbidity and in-patient care is strong. The question of whether the adverse features related to catheter use, such as catheter thrombosis and bacteraemia, are specifically related to use of a catheter or are simply related to the greater level of comorbidity expressed by the population who require catheter insertion has been subject to controversy. This question is addressed in chapters 2 and 3 of this thesis where prevalent and incident RRT populations are studied by detailed univariate and multivariate analyses in an effort to determine the relative strength of independent association between clinical, laboratory and vascular access variables and the outcomes of catheter-related bacteraemia and thrombosis.

Methods of preventing and treating CVC related bacteraemia and thrombosis have come under close scrutiny. Numerous strategies targeting these problems have been

examined however the introduction of anticoagulant-antimicrobial catheter lock solutions into clinical practice appears to have been successful despite many uncertainties remaining. In this thesis the contentious issue of heparin and also antibiotic-lock solution leakage into the circulation is addressed by the work detailed in Chapter 4. This work describes a randomised controlled study of heparinised catheter lock solutions and their relative effects on systemic markers of coagulation. Secondly, the uncertain issue of how the combination of an antimicrobial with an anticoagulant may affect the antimicrobial performance of the antibiotic lock solution is addressed in the studies detailed in Chapter 5. This work describes an in-vitro assessment of the properties exhibited by heparin and vancomycin against planktonic and biofilm embedded staphylococci.

Many of the complications of vascular access, however, can only be definitively addressed by removal or replacement of the vascular access device. As noted earlier, significant controversy exists as to the best means of assessing the central veins in complex patients. In recent years the development of contrast-enhanced MRV imaging had become a favoured approach. The work detailed in Chapter 6 describes a retrospective assessment of all CE-MRV examinations undertaken in haemodialysis patients within the renal unit of Glasgow Royal Infirmary. This work describes the abnormalities found on CE-MRV and the subsequent clinical course followed by the patients in whom CE-MRV imaging was undertaken. It was during this study that a relatively high prevalence of the serious condition nephrogenic systemic fibrosis was noted. During this period the first published association between NSF and gadolinium-enhanced MRV was made (77). Consequently a large retrospective analysis of all RRT patients in the West of Scotland was made to assess whether an

association between gadolinium contrast agents and NSF existed in our population.

This work is presented in Chapter 7.

Chapter 2

A Retrospective Study of Vascular Access in Haemodialysis

Patients and Risk of Bacteraemia and Death

2.1 Background

Haemodialysis patients have high rates of morbidity and mortality of which infection makes a significant contribution (7, 78). The immunosuppressive effects of advanced renal failure, concurrent co-morbid disease and associated malnourishment combine with the repeated intravascular intervention required for haemodialysis to provide an environment conducive to the development of bacteraemia and sepsis syndrome (79).

The effect of sepsis can be profound. The financial cost of treating dialysis patients with septicaemia is high with estimates of up to \$32,000 per patient hospitalised with bacteraemia in the United States (80-84). Septicaemia in a haemodialysis patient confers a relative risk for mortality of 2.8 with relative risks of subsequent myocardial infarction, cardiac failure and stroke of 4.1, 5.5 and 4.1 respectively. The development of sepsis syndrome is now classed as second only to cardiovascular disease as the leading cause of death in patients on renal replacement therapy (RRT) (78). These implications have become increasingly recognised and recent reports have suggested methods such as cohort surveillance and bacteraemia monitoring to help define at risk groups before targeting resources to prevent and treat bacteraemia (85, 86).

Vascular access is an established risk factor for sepsis in patients on RRT. Different types of haemodialysis vascular access are associated with differing rates of bacteraemia (87-91). Patients using different types of vascular access, however, tend to have different clinical characteristics. Indeed, the true independence of the association between vascular access and bacteraemia in comparison with other

clinical and laboratory risk factors has yet to be clearly established. This study was designed to determine which routinely recorded clinical variables, laboratory variables and vascular access types are *independently* associated with bacteraemia and death in haemodialysis patients.

2.2 Methods

A retrospective analysis of all patients in our renal unit who were on haemodialysis on the 1st of January 2004 was performed. Clinical, demographic and laboratory variables for each patient were retrieved from the unitary electronic patient record. Where multiple measurements of a single variable had been recorded, the first value after 1st January 2004 was used.

Clinical variables collected were age, gender, primary renal diagnosis, the presence or absence of diabetes, length of time on renal replacement therapy, vascular access flow rate on haemodialysis and urea reduction ratio (a standard measure of haemodialysis adequacy). The dialysis access in use at the time of study initiation was recorded as one of four categories: native arteriovenous fistula (AVF) – a surgically created anastomosis between artery and vein to create a robust port of access for haemodialysis, synthetic vascular access graft, tunnelled central venous catheter (TCVC) and non-tunnelled central venous catheter (NTCVC).

Laboratory variables collected at study entry were haemoglobin, serum C-reactive protein (CRP), serum ferritin, serum albumin, serum adjusted calcium and phosphate

product, serum alkaline phosphatase, serum parathyroid hormone concentration and total serum cholesterol to high-density lipoprotein (HDL) cholesterol ratio.

Outcomes

Outcomes were ascertained over an 18-month period of follow up terminating on 1st of July 2005. Bacteraemia events were determined by analysis of all positive in-patient and out-patient blood culture results from the renal unit reported by the bacteriology laboratory during the period of study in conjunction with analysis of the patient's clinical notes and electronic patient record. Bacteraemia was regarded as significant if positive blood cultures were associated with a raised systemic inflammatory response (e.g. pyrexia, raised CRP, raised white-cell count). Sub-clinical bacteraemia was not evaluated. This approach is in keeping with the consensus definition of clinically significant bacteraemia and consistent with that used in routine clinical practice. Where patients were found to have developed a significant bacteraemia, the date of the first positive blood culture result was entered as the event date and the time to event subsequently calculated. The patient was then removed from further bacteraemia analysis. This study did not examine cases of recurrent bacteraemia and thus was a 'time to first bacteraemia' analysis. In all cases of confirmed bacteraemia the causative organism was recorded and sensitivity profiles examined. Patients who remained on haemodialysis throughout the observation period and did not develop bacteraemia had a census date of 1st July 2005 recorded.

All patients who died during the follow up period had the date of death recorded as an endpoint. All patients who were discharged, were transferred to another

haemodialysis unit or who switched renal replacement modality were assigned a census date corresponding to the date of discharge from the haemodialysis cohort.

Our standard unitary protocol for catheter care was employed throughout the observation period. Specifically, this demanded complete sterile barrier precautions during catheter insertion and when manipulating the catheter hub. Following catheter-hub manipulation, the skin surrounding the insertion site was soaked with chlorhexidine solution prior to a sterile dressing being applied. All haemodialysis patients were on a standard regimen of 3 haemodialysis sessions per week, each of minimum 4 hours per session with subsequent increases in session length up to 5 hours in order to achieve a target urea-reduction ratio of 70%. This conforms to UK Renal Association Guidelines. Water quality in the haemodialysis facilities was quantified on a pass/fail basis using a failure threshold of 0.25 endotoxin units per mL with pass rates for all reverse osmosis units in the unit typically averaging 98% with total viability counts averaging <10 cfu/mL.

Analysis

Sample size was determined by the size of the haemodialysis population within Glasgow Royal Infirmary Renal Unit on 01/01/2004. No other similar patient populations were available for study in whom reliable data retrieval could be achieved. Statistical analysis was performed by SPSSTM version 14.0 (SPSS Inc, IL, USA). Normality testing was performed on all of the recorded continuous variables. Student's t-testing and Mann-Whitney U testing were then used to assess differences between TCVC and NTCVC groups with AVF as a comparator.

Bacteraemia free survival and mortality rates for each access type was subject to Kaplan-Meier survival analysis and Log Rank testing with $p < 0.05$ being regarded as statistically significant. The remaining variables were subject to univariate analysis with Students t-testing and Mann-Whitney U testing as appropriate to test for associations between characteristics at study entry and the development of bacteraemia and mortality. The relationship between bacteraemia and subsequent mortality was subject to Pearson Chi-square testing. After application of the bonferroni correction a p-value of < 0.0025 was regarded as statistically significant in these univariate analyses. Multivariate analysis was performed using a Cox proportional hazards model with a stepwise conditional method of analysis to test for: 1) an independent association with the development of bacteraemia and 2) an independent association with mortality. To avoid over fitting of the multivariate models the convention of limiting the number of independent variables entered to approximately 10% of the number of outcome events was followed. In our analysis independent variables for entry into the models were selected according to their p-values on univariate testing. On multivariate analysis all reported p-values < 0.05 were regarded as significant.

2.3 Results

A search of the renal unit electronic patient record was conducted to identify all patients who underwent haemodialysis between 1st and 3rd of January 2004. A total of 265 patients were identified of whom 136/265 (51.3%) were male. Mean age was

63.5 years (SD 14.6) whilst median duration of renal replacement therapy prior to 1st of January 2004 was 1076 days (range 7-12,829). All patients were found to have established renal failure with none of the cohort on dialysis for acute renal failure. 59/265 (22.3%) of the cohort had a diagnosis of diabetes mellitus. At study entry 206/265 (77.7%) were dialysing via native AVF, 31/265 (11.7%) were dialysing through a TCVC, 26/265 (9.8%) were dialysing through a NTCVC and 2/265 (0.8%) were dialysing through a synthetic vascular graft. As only two patients were dialysing through a synthetic graft, they were not subject to further analysis. Table 2.1 displays the characteristics of the full cohort whilst table 2.2 compares the clinical and laboratory characteristics for each vascular access type.

Variable	Data Values
Age (years)	63.5 (14.6)*
Male	136/265 (51.3%)
Female	129/265 (48.7%)
Diabetes	59/265 (22.3%)
Duration of RRT (days)	1076 (376, 2095)**
Haemoglobin (g/dL)	11.3 (1.8)*
Ferritin (ng/ml)	356 (196, 568)**
Albumin (g/L)	37.5 (4.8)*
C-reactive protein (mg/L)	17.4 (2.9)*
Alkaline phosphatase (U/L)	203 (156, 304)**
Parathyroid Hormone (pmol/L)	22.0 (11.0, 40.0)**
Calcium x phosphate product	4.08 (1.34)*
Cholesterol:HDL	3.39 (1.24)*
Haemodialysis blood flow (mL/min)	300.0 (59.4)*
Urea reduction ratio (%)	71.3 (8.9)*

Table 2.1 – Data values and descriptive statistics for the laboratory variables studied in the full cohort. Data values are expressed as value (%), *mean (SD) or **median (Q1, Q3).

	Arteriovenous Fistula (n=206)	Tunnelled Catheter (n=31)		Non-Tunnelled Catheter (n=26)	
Clinical Features					
Age (years)	63.8 (14.4)*	63.5 (15.2)*	$p = 0.90$	62.8 (17.0)*	$p = 0.75$
Male	111 (53.9%)	10 (32.3%)	$p = 0.03$	14 (53.8%)	$p = 0.99$
Female	95 (46.1%)	21 (67.7%)	$p = 0.03$	12 (46.2%)	$p = 0.99$
Diabetes	43/206 (20.9%)	8/31 (25.8%)	$p = 0.49$	7/26 (26.9%)	$p = 0.46$
Duration on RRT	1138 (412, 2048)**	1511 (765, 5288)**	$p = 0.01$	278 (55, 903)**	$p < 0.001$
Haemodialysis blood flow (ml/min)	309 (56.5)*	267 (60.9)*	$p < 0.001$	268.8 (58.6)*	$p = 0.001$
Urea reduction ratio (%)	71.9 (8.9)*	70.45 (8.04)*	$p = 0.27$	66.7 (8.73)*	$p = 0.01$
Primary Renal Diagnosis					
Primary Glomerulonephritis	39/206 (18.9%)	6/31 (19.4%)	$p = 0.96$	6/26 (23.1%)	$p = 0.61$
Interstitial Nephropathies	57/206 (27.7%)	10/31 (32.2%)	$p = 0.60$	5/26 (19.2%)	$p = 0.36$
Multisystem Disease	28/206 (13.6%)	2/31 (6.5%)	$p = 0.27$	2/26 (7.7%)	$p = 0.40$
Diabetic Nephropathy	33/206 (16.0%)	4/31 (12.9%)	$p = 0.66$	8/26 (30.8%)	$p = 0.06$
Laboratory Variables					
Haemoglobin (g/dL)	11.4 (1.7)*	11.6 (1.98)*	$p = 0.74$	10.2 (1.56)*	$p = 0.001$
Ferritin (ng/ml)	357 (200.0, 578.0)**	467 (192, 575)**	$p = 0.94$	253 (88.5, 432.5)**	$p = 0.06$
Albumin (g/L)	37.9 (5.0)*	36.6 (3.13)*	$p = 0.007$	35.7 (4.1)*	$p = 0.005$
C-reactive protein (mg/L)	15.7 (2.9)*	23.9 (3.0)*	$p = 0.06$	24.5 (2.7)*	$p = 0.03$
Calcium x phosphate	4.13 (1.36)*	4.03 (1.18)*	$p = 0.87$	3.8 (1.5)*	$p = 0.20$
Alkaline phosphatase (U/ml)	196 (156, 297)**	220 (154.5, 333.0)**	$p = 0.43$	239 (153.0, 466.0)**	$p = 0.41$
Parathyroid hormone (pmol/L)	35.5 (38.7)*	29.7 (29.8)*	$p = 0.37$	24.7 (22.4)*	$p = 0.23$
Chol:HDL	3.5 (1.3)*	2.73 (0.58)*	$p = 0.03$	3.2 (1.0)*	$p = 0.34$

Table 2.2 - Characteristics for each of the main vascular access groups. All statistical testing of the tunnelled and non-tunnelled catheter groups was carried out in comparison with the characteristics of the arteriovenous fistula group. Data values are expressed as value (%), *mean (SD) or **median (Q1, Q3).

As expected, patients dialysing through a TCVC had a significantly lower haemodialysis blood flow rate when compared to those dialysing through an AVF. Otherwise, those using a TCVC demonstrated trends towards lower serum albumin, lower serum cholesterol to HDL ratios and longer median duration on RRT in comparison to those dialysing through an AVF. 26/31 patients were dependent on their TCVC as their only remaining option for vascular access whilst 5/31 patients were using a TCVC prior to definitive vascular access being created.

Patients dialysing through a NTCVC had significantly lower haemoglobin and lower haemodialysis blood flow rates than those dialysing through either a TCVC or AVF. 14/26 (53.7%) patients had been on dialysis for >3 months and had temporary vascular access problems that had required NTCVC insertion. 12/26 (46.2%) had started RRT for established renal failure within 3 months of study entry. This proportion accounts for the significantly shorter duration on RRT in the NTCVC group when compared with the TCVC and AVF groups ($p=0.001$). None of these patients had acute renal failure (i.e. recovered renal function within a 3 month period). Patients dialysing through a NTCVC had trends towards lower albumin and urea-reduction ratios as well as higher CRP compared to the AVF group.

During the observation period, 15 patients underwent renal transplantation, 5 transferred to other renal units, 2 patients switched to continuous ambulatory peritoneal dialysis (CAPD) and 1 patient recovered renal function and was subsequently discharged.

Bacteraemia

45/263 (17.1%) patients developed at least one episode of bacteraemia over the 18-month period. 39/45 (86.7%) of cases were secondary to staphylococci of which 15/45 (33.3%) cases were due to coagulase-negative staphylococci and 5/45 (11.1%) cases were due to methicillin-resistant staphylococcus aureus (MRSA) infection. 3/45 (6.7%) cases were due to gram-stain negative bacteria and 3/45 (6.7%) were secondary to other bacterial subspecies. The relative frequencies of cases of catheter-related bacteraemia from each pathogenic group are demonstrated in figure 2.1.

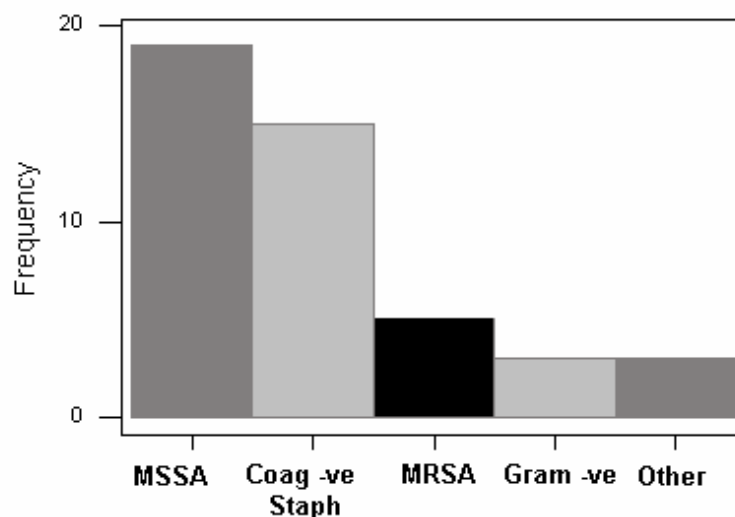


Figure 2.1 – Frequency plot of the types of pathogenic organisms in cases of clinically significant bacteraemia demonstrated over the 18-month period of observation.

Analysis of bacterial resistance profiles demonstrated that 9/13 (69%) cases of the coagulase-negative staphylococci where antibiotic sensitivities were available, were resistant to the antibiotic flucloxacillin. Of the 45 patients who developed bacteraemia 17 patients (37.8%) died during the 18-month observation period.

Univariate analysis was performed on the laboratory and clinical variables recorded at the start of the study period to examine whether there was an association with the development of bacteraemia. The actuarial 18 month bacteraemia-free survival in the cohort dialysing via AVF at study entry was significantly higher than the cohorts dialysing through TCVC or NTCVC (88.8% v 54.8% v 69.2% respectively; $p<0.001$). This is demonstrated graphically as a Kaplan-Meier plot in figure 2.2.

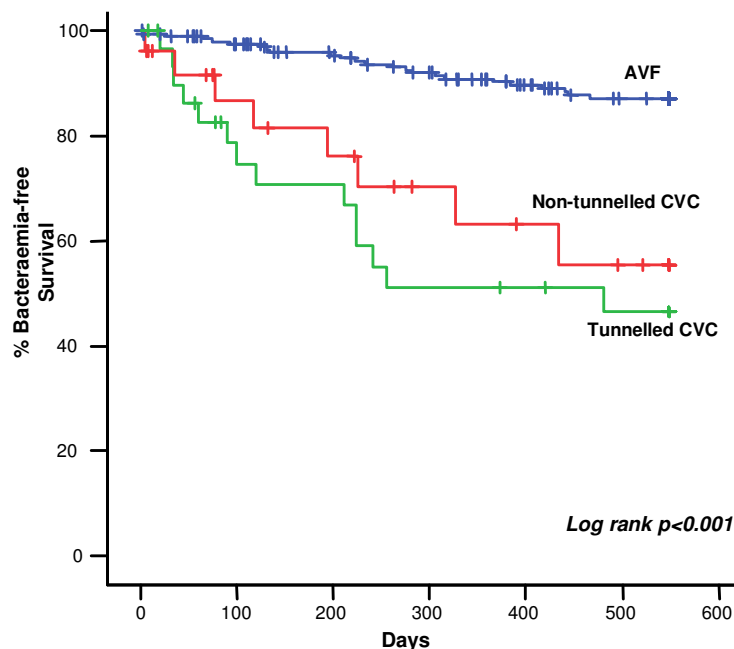


Figure 2.2 – Kaplan-Meier plot of time to bacteraemia per haemodialysis vascular access type. ‘AVF’ = arteriovenous fistula, ‘Tunnelled CVC’ = tunnelled central venous catheter, ‘Non-tunnelled CVC’ = non-tunnelled central venous catheter.

No significant difference in bacteraemia event rates was found when directly comparing TCVCs with NTCVCs ($p=0.29$). Patients who developed bacteraemia were found to have trends towards higher CRP levels and lower serum albumin levels at study entry than their bacteraemia free counterparts but this did not reach the bonferroni corrected significance

level. Age (bacteraemia 62.9yrs v no-bacteraemia 63.8yrs, $p=0.74$) and the presence of diabetes (bacteraemia 8/45 (17.8%) v no-bacteraemia 34/218 (15.6%), $p=0.66$) were not associated with bacteraemia.

Multivariate analysis demonstrated hazard ratios (HR) for the development of bacteraemia in patients dialysing with TCVCs and NTCVCs of 5.43 (95% CI 2.67-11.0, $p<0.001$) and 3.14 (95% CI 1.32-7.48, $p=0.01$) respectively compared to those patients dialysing through an AVF. There was also an independent association between elevated CRP at study entry and the risk of developing bacteraemia over an 18-month period with a HR of 1.49 (95% CI 1.12-1.98, $p=0.006$). The results of the univariate and multivariate analyses for the outcome of bacteraemia are demonstrated in table 2.3.

Variable at Study Entry		Univariate Analysis		Multivariate Analysis	
		Data Values	p-value	Hazard Ratio (95% CI)	p-value
Tunnelled Catheter (n=31)	Bacteraemia	14 (45.2%)	$P<0.001^a$	5.43 (2.67, 11.0)	$p<0.001$
	No Bacteraemia	17 (54.8%)			
Non-tunnelled Catheter (n=26)	Bacteraemia	8 (30.8%)	$P=0.006^a$	3.14 (1.32, 7.48)	$p=0.01$
	No Bacteraemia	18 (69.2%)			
CRP (mg/L)	Bacteraemia	25.3 (2.9)*	$P=0.015^b$	1.49 (1.12, 1.98)	$p=0.006$
	No Bacteraemia	16.0 (2.9)*			
Albumin (g/L)	Bacteraemia	36.0 (4.9)*	$P=0.021^b$	-	-
	No Bacteraemia	37.9 (4.7)*			
HD Flow (ml/min)	Bacteraemia	283 (56)*	$P=0.036^b$	-	-
	No Bacteraemia	303 (60)*			

Table 2.3 – Factors that underwent both univariate and multivariate analysis and their association with the incidence of bacteraemia. Data values are expressed as value (%) or *mean (SD) where appropriate. ‘CRP’ = C-reactive protein, ‘HD’ = haemodialysis, ^a Pearson Chi-Square testing, ^b Student's t-test.

Mortality

65/263 (24.7%) patients died during the follow-up period and were found to have significantly elevated serum CRP, low serum albumin and increased age at study entry compared with those who survived. The actuarial 18 month patient survival in the cohort dialysing via AVF at study entry was better than the cohorts dialysing through TCVC or NTCVC (79.1% v 64.5% v 57.7% respectively; $p<0.019$). This is demonstrated graphically as a Kaplan-Meier plot in figure 2.3.

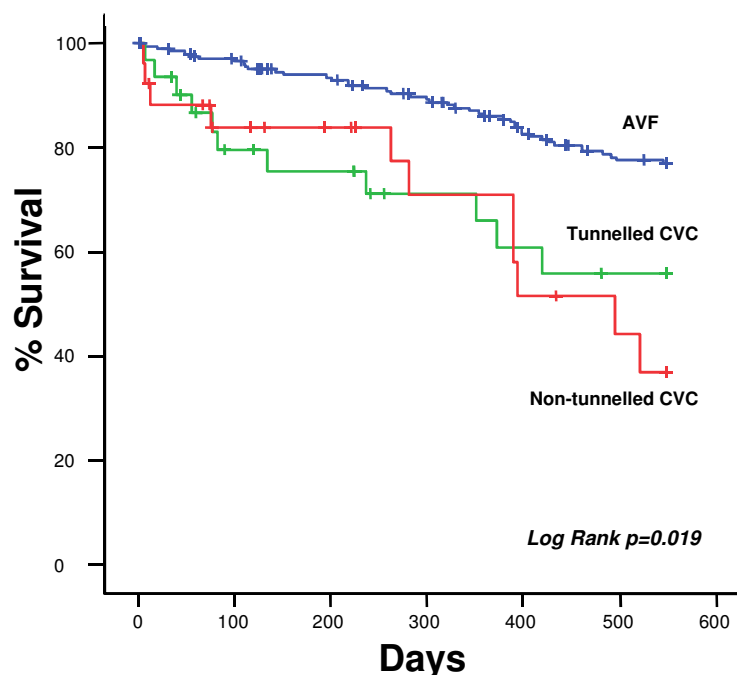


Figure 2.3 – Kaplan-Meier survival plot of mortality per haemodialysis vascular access type. ‘AVF’ = arteriovenous fistula, ‘Tunnelled CVC’ = tunnelled central venous catheter, ‘Non-tunnelled CVC’ = non-tunnelled central venous catheter.

Trends of association with mortality by univariate analysis were seen with elevated serum alkaline phosphatase levels and lower levels of haemoglobin and the product of serum calcium and phosphate at study entry. A diagnosis of diabetes mellitus was found to be

associated with mortality (death occurring in 21/58 (36.2%) diabetics and 44/205 (21.5%) of non-diabetics, $p=0.02$) as was a diagnosis of dialysis related bacteraemia (death occurring in 17/45 (37.8%) bacteraemics and 48/218 (22.0%) of non-bacteraemics, $p=0.026$).

Multivariate analysis demonstrated an independent association with death with the use of TCVCs and NTCVCs (HR 2.75; $p=0.012$ and HR 3.39; $p=0.001$ respectively) compared with AVFs, low serum albumin (HR 0.92, 95% CI 0.87-0.97, $p=0.003$), elevated alkaline phosphatase (HR 1.002, 95% CI 1.000-1.003, $p=0.011$) and increasing age (HR 1.04, 95% CI 1.02-1.07, $p<0.001$). The results of the univariate and multivariate analyses for the outcome of mortality are demonstrated in table 2.4.

Variable at Study Entry		Univariate Analysis		Multivariate Analysis	
		Mean (SD)	p-value	Hazard Ratio (95% CI)	p-value
Non-tunnelled Catheter	Dead	11/26 (42%)	$p=0.015^a$	3.39 (1.67, 6.87)	$p=0.001$
Tunnelled Catheter	Dead	11/31 (35%)	$p=0.016^a$	2.75 (1.25, 6.04)	$p=0.012$
Age (Years)	Dead Alive	68.6 (13.2)* 62.0 (14.7)*	$p=0.001^b$	1.04 (1.02, 1.07)	$p<0.001$
Alk phos (U/L)	Dead Alive	221 (107, 976)** 192 (58, 943)**	$p=0.003^c$	1.002 (1.000, 1.003)	$p=0.011$
Albumin (g/L)	Dead Alive	35.8 (4.8)* 38.1 (4.6)*	$p<0.001^b$	0.92 (0.87, 0.97)	$p=0.003$
CRP (mg/L)	Dead Alive	27.2 (2.8)** 14.6 (2.8)**	$p<0.001^b$	-	-

Table 2.4 – Factors that underwent both univariate and multivariate analysis and their association with the incidence of mortality. Data values are expressed as value (%), *mean (SD) or **median (Q1, Q3). ‘Alk Phos’ = Alkaline Phosphatase, *Median (Q1, Q3), **Geometric Mean (SD), ^a Pearson Chi-Square testing, ^b Student's t-test, ^c Mann-Whitney U test.

2.4 Discussion

This study examined bacteraemia and mortality in a cohort of haemodialysis patients with established renal failure that had a pattern of vascular access which was representative of a typical UK chronic haemodialysis population. The rates of bacteraemia (17.1%) and mortality (24.7%) over the 18-month period of observation equate to those described in the literature to date (91). Through a retrospective methodology an independent association between several commonly recorded clinical and laboratory variables and subsequent outcome was demonstrated.

Of particular note was the finding that use of synthetic vascular access catheters had an especially strong association with risk of bacteraemia and death, an effect that was independent of known adverse factors such as age, sex, diabetes, anaemia, and the other clinical and laboratory variables that were studied and found to be not to be significantly associated with outcome on univariate testing (92). Whilst tunnelled catheters were found to be used in patients who had been on dialysis longer, we found this to be a product of patients whose peripheral vasculature had been exhausted of attempts to create an arteriovenous fistula or graft. None of the TCVC group had been exposed to bacteraemia in, at least, the period 3 months prior to commencement of the study. Indeed, the demographics and co-morbidities of the tunnelled catheter group and arteriovenous fistula group were very similar and thus the conclusion that adverse outcomes are independently associated TCVC use when compared to AVFs would appear to be accurate. When considering the NTCVC group, it was evident that this consisted of a higher proportion of patients who had commenced RRT for established renal failure within three months of study entry and in this setting the co-morbidities that often arise during this period may

contribute to the difference in clinical outcomes seen in this particular group compared to the others. No difference in bacteraemia rates was seen between NTCVCs and TCVCs although higher mortality rates were seen in the NTCVC group. It is difficult to draw any meaningful conclusions when comparing these groups due to the inherent limitations of the methodology employed.

The limitations incurred by the retrospective study design and subsequent extrapolation of a 'snapshot' of vascular access related to long-term outcome have to be acknowledged. In particular, this method of analysis was not sensitive to potential changes in vascular access type over the period of observation. Many patients using NTCVCs would be reasonably expected to migrate onto TCVCs and then AVFs as they progress through the early months following commencement on RRT. One would, however, expect that this potential confounding would weaken any association between vascular access and clinical outcome since risks presumably lower when patients convert from central venous catheter to arteriovenous fistulae. Nonetheless, in such an observational study it must be remembered that the primary drive behind access choice was from the clinician responsible for each patient's care. Therefore, whilst access type has been associated with adverse events it should be assumed that each patient was on the optimal vascular access possible within their clinical situation. Another limitation was the lack of detailed data on comorbidity, other than the data we included regarding age, duration on RRT and diabetes. Whilst these variables will account for some of the main comorbidities experienced in this population, prospective study incorporating comorbidity-scored data would be of great benefit in bringing clarity to this perpetual confounder of vascular access studies.

Sample size was also a significant limitation in this study, especially when attempting to relate these results to the haemodialysis population within the UK. As an example, the current UK haemodialysis population is approximately 20,000 patients. To determine a hazard ratio with 95% confidence interval of 1.00 to 1.05 and significance level of $p < 0.05$ in such a large population size would require a sample size of over 8000 patients. Clearly the sample size in this study falls short of such levels and this is reflected in the wide confidence intervals demonstrated when considering the hazard ratios in each of the access groups with both outcome measures.

The significant role of transcutaneous vascular access devices in increasing risk of bacteraemia was further implicated by the finding that 86.7% of bacteraemia was secondary to staphylococci. Within this cohort methicillin-resistant staphylococcus aureus (MRSA) accounted for 11.1% of all bacteraemia. These rates are consistent with those published in the current literature where the vast majority of clinically significant infection in dialysis patients has been found to be secondary to staphylococcal sub-species derived from the surface of the skin (92-96).

Not only is the identity of the causative organisms important, but the spectrum of antibiotic resistance is also of importance when determining appropriate first line antibiotic treatment (96). Sub-analysis of the cohort demonstrated that two-thirds of all coagulase negative staphylococci isolated on blood cultures were flucloxacillin resistant. This, in conjunction with an 11.1% prevalence of MRSA would suggest that current antibiotic protocols that use flucloxacillin as first line therapy may require re-consideration with regards local patterns of anti-microbial resistance.

The finding of an independent association between CRP at study entry and the development of subsequent bacteraemia, an association that remained after adjusting for several potential confounders on multivariate testing, was also of interest. The exact nature of the relationship between infection, inflammation and outcome remains to be clarified. When considering inflammatory burden and mortality, several studies have demonstrated a significant association between increased inflammatory indices and heightened mortality rates, possibly through a process of vascular endothelial dysfunction, subsequent atherogenesis and a resultant tendency towards cardiovascular events (89, 97-100). Other potential drivers of CRP release in haemodialysis patients include pre-existent, latent infection, underlying inflammatory conditions such as connective tissue disease, vasculitis and glomerulonephritis, and exposure to inflammatory triggers inherent with the haemodialysis process. These include exposure to invasive procedures to assess the vasculature and establish vascular access, repeated exposure of blood to the extracorporeal circuit and exposure to low levels of endotoxin through dialysis water. Such is the prevalence of vascular disease in the haemodialysis population, however, the link between CRP and underlying vascular endothelial damage is an attractive target to those keen to explore how CRP levels translate into increased numbers of cardiovascular and other adverse events. Not all cardiovascular mortality in dialysis patients can be explained by traditional risk factors (97) and indeed sepsis and inflammatory burden could be plausible pre-disposing factors in many cardiovascular deaths (96). Proving causation is, however, difficult given the wide variety of confounders that would need accounted for.

In summary, this work demonstrates the prevalence of bacteraemia and mortality in a UK haemodialysis unit and provides an assessment of potential risk association across a spectrum of laboratory and clinical variables that is broader than most similar studies.

Many previous studies have been conducted in US populations which are demographically different and function through a different system of health care delivery to that of the UK population. What this study therefore adds to the field is the demonstration of similar rates of bacteraemia and mortality alongside a similar profile of vascular access risk association in a UK population. Similarly, the strength of association found with haemodialysis vascular access type compared to other routinely recorded clinical and laboratory variables is clearly demonstrated and comparable to that of other studies in different populations. Of additional relevance and interest is the degree to which vascular access type translates into adverse risk compared with other variables, as is the finding of an association between baseline CRP and subsequent bacteraemia risk. Whilst of interest, all these findings must be tempered by the inherent limitations of the study design, as noted above.

2.5 Conclusion

These data found that use of synthetic vascular access catheters had an especially strong association with risk of bacteraemia and death in a typical haemodialysis cohort, an effect that was independent of age, sex, diabetes, anaemia, and the other clinical and laboratory variables that were studied.

Chapter 3

A Prospective Observational Study of Catheter-related Bacteraemia and Thrombosis in a Haemodialysis Cohort: Univariate and Multivariate Analyses of Risk Association

3.1 Background

The work detailed in Chapter 2 demonstrated that central venous catheters compare poorly with arteriovenous fistulae when considering bacteraemia and mortality in patients on haemodialysis. A strong, independent association with poor outcome was demonstrated after accounting for several known markers of comorbidity despite the limitations inferred by a retrospective study design not sensitive to changes in vascular access.

Central venous catheters are, however, a fundamental component in delivering haemodialysis. They may be inserted quickly and without use of surgical support. Central venous catheters are thus especially useful when patients require dialysis urgently and have no pre-existent functioning vascular access. Alternatively, catheters may be used in patients in whom sites for establishing arteriovenous fistulae or grafts have been exhausted.

The two most common complications of catheterisation are catheter thrombosis and catheter-related bacteraemia (101). Developing either of these complications incurs significant impact on both the functionality and lifespan of the catheter and confers high rates of morbidity and mortality notwithstanding considerable cost (82-84, 102). The work in Chapter 2 highlighted some of the different demographics between patients using each of the vascular access types, differences that may change further when patients with acute renal failure are included. Whether the complication profile seen in patients using catheters is a result of a higher burden of comorbidity seen in selected groups of complex patients is yet to be conclusively demonstrated.

To test this hypothesis in more detail, the work in this chapter examines rates of bacteraemia and thrombosis experienced with all types of vascular access catheter insertion conducted in an incident renal replacement therapy (RRT) cohort over a 2-year period of prospective observation. Univariate and multivariate analyses were undertaken to identify and quantify *independent risk association* between clinical variables, laboratory variables and measures of comorbidity, with regard to both catheter thrombosis and catheter-related bacteraemia.

3.2 Methods

A prospective analysis of all incident vascular access haemodialysis catheter insertions over the period starting 05/08/2005 and ending 05/08/2007 was performed. The date of each catheter insertion was recorded along with the type of catheter inserted, the anatomical location of catheter insertion and whether the catheter insertion was conducted as a catheter-exchange procedure over a guidewire. Clinical casenotes and our unitary electronic patient record were used to obtain details of a number of clinical and laboratory variables that were routinely collected as part of our standard care, on the date of catheter insertion.

Clinical variables including age, sex and concurrent antibiotic, anticoagulant, immunosuppressive and statin therapy were recorded. Modified Charlson comorbidity scores (103-105), a diagnosis of diabetes, cause and duration of renal failure, body mass index, systolic blood pressure, diastolic blood pressure and haemodialysis blood flow on dialysis immediately following catheter insertion (as determined by dialysis machine blood

pump speed) were also obtained. Laboratory variables including haemoglobin, platelet count, neutrophil count, lymphocyte count, CRP, albumin, adjusted calcium, phosphate and urea reduction ratio prior to catheter insertion were recorded.

Outcomes

Patients were prospectively followed up to the point of catheter removal or the cessation of the study. During this period two main outcomes were assessed: catheter-related bacteraemia (CRB) and catheter failure with removal due to poor haemodialysis blood flow as a surrogate of catheter thrombosis. All patients who died or were discharged to another renal unit with a central venous catheter in-situ were assigned a census date corresponding to the date of discharge from the renal unit. Patients who remained on haemodialysis at the end of the observation period with a central venous catheter in-situ had a census date of 5th August 2007 recorded.

CRB events were sought through analysis of all positive blood culture results from the renal patient population as reported by the bacteriology laboratory in conjunction with analysis of the patient's clinical notes and electronic patient record. CRB was defined as the presence of positive blood cultures associated with a raised systemic inflammatory response (e.g. pyrexia, raised CRP, raised white-cell count) and the absence of clinical or radiological signs of a non-catheter related source of infection. Sub-clinical bacteraemia was not evaluated. This approach is in keeping with the definition of CRB commonly reported in the literature and consistent with that used in routine clinical practice. Where patients were found to have developed CRB, the date of the first positive blood culture

result was entered as the event date and the time to event subsequently calculated. Catheter removal due to poor flow was defined as removal of a central venous catheter in response to low haemodialysis blood flows that consistently impaired effective haemodialysis delivery despite optimal anticoagulation and/or thrombolytic intervention. This reflected routine clinical practice in the participating renal unit. All decisions regarding catheter removal were made by the clinical team responsible for each patient's care and were made independently from the investigator. The standard unitary protocol for catheter care was employed throughout the observation period. Specifically, this demanded complete sterile barrier precautions during catheter insertion and when manipulating the catheter hub. Following catheter-hub manipulation, the skin surrounding the insertion site was soaked with chlorhexidine solution prior to a sterile dressing being applied. Between haemodialysis sessions all catheters were 'locked' with 5000iu/mL heparin of volume consistent with the manufacturer's stated catheter luminal volume.

All haemodialysis patients were on a standard regimen of 3 haemodialysis sessions per week, each of minimum 4 hours per session with subsequent increases in session length up to 5 hours in order to achieve a target urea-reduction ratio of >65%.

Analysis

Statistical analysis was performed using SPSSTM version 14.0 (SPSS Inc, IL, USA). Parametric testing with student's t-test and non-parametric testing with the Wilcoxon-sign rank test were used in the assessment of continuous variables where appropriate. Categorical variables were assessed using Pearson Chi-square testing. Rates of

bacteraemia free survival and catheter removal due to poor haemodialysis flow were compared by Kaplan-Meier survival analysis with log-rank testing with event rates expressed per 1000 catheter days. When performing the univariate analyses, in view of the large number of variables assessed, Bonferroni's correction was applied with a significance level of $\alpha \leq 0.0025$.

Multivariate analysis was performed using a Cox proportional hazards model with a stepwise conditional method of analysis to test for: 1) an independent association with the development of catheter-related bacteraemia and 2) an independent association with catheter failure due to poor haemodialysis blood flow. To avoid over fitting of the multivariate models the convention of limiting the number of independent variables entered to approximately 10% of the number of outcome events was followed. In our analysis independent variables for entry into the models were selected according to their p-values on univariate testing. On multivariate analysis p-values ≤ 0.05 were regarded as significant.

3.3 Results

Over the 2-year period a total of 365 patients underwent 823 central venous catheter insertions. Patients were of median age 66.4yrs (range 19.8, 87.1yrs) with 203/365 (55.6%) male. 130/365 (35.6%) patients were found to have acute renal failure (ARF) defined as a recovery of renal function with cessation of dialysis within 90 days. 60/365 (16.4%) patients underwent catheter insertion in the context of acute on chronic renal failure (A/CRF) whilst 175/365 (47.9%) patients underwent catheter insertion in the context of chronic renal failure (CRF). Clinical and laboratory variables recorded at the point of catheter insertion are detailed in table 3.1.

Variable	TCVC	NTCVC IJug	NTCVC Fem	NTCVC IJug (G)	NTCVC Fem (G)
Patients	N=301	N=313	N=136	N=60	N=13
CRB events	N=72	N=25	N=8	N=8	N=2
Poor Flow events	N=40	N=49	N=27	N=12	N=3
ARF	20 (6.6%)	109 (34.8%)	47 (34.6%)	35 (58.3%)	3 (23.1%)
A/CRF	21 (7.0%)	63 (20.1%)	23 (16.9%)	10 (16.7%)	1 (7.7%)
CRF	260 (86.4%)	141 (45.0%)	66 (48.5%)	15 (25.0%)	9 (69.2%)
Diabetes	129/301 (42.9%)	116/313 (37.1%)	48/136 (28.9%)	22/60 (36.7%)	5/13 (38.5%)
Statin	183/301 (60.8%)	150/313 (47.9%)	61/136 (44.6%)	22/60 (36.7%)	7/13 (53.8%)
Anticoagulation	231/301 (76.7%)	182/313 (58.1%)	83/136 (61.0%)	36/60 (60.0%)	10/13 (76.9%)
Immunosuppression	32/301 (10.6%)	35/313 (11.2%)	11/136 (8.1%)	7/60 (11.7%)	1/13 (7.7%)
Antibiotic	294/301 (97.7%)	128/313 (40.9%)	62/136 (45.6%)	32/60 (53.3%)	7/13 (53.8%)
Modified Charlson score	2 (0,13) **	2 (0,8) **	2 (0,9) **	1 (0,4) **	1.5 (0,8) **
Days on RRT	531 (0,14061) **	9 (0,13690) **	4 (0,8653) **	5 (0,3484) **	482 (0,2551) **
Catheter days	92 (1,692) **	9 (0,200) **	5 (0,34) **	7 (0,97) **	6 (0,32) **
Urea reduction ratio	66.1 (11.5) *	68.4 (9.9) *	67.2 (7.2) *	65.4 (5.4) *	62.6 (10.0) *
Dialysis blood flow (mls/min)	300 (58, 350) **	250 (120,375) **	220 (100,350) **	200 (150,330) **	250 (160,400) **
SBP (mmHg)	130 (28) *	137 (27) *	130 (28) *	135 (28) *	145 (20) *
DBP (mmHg)	74 (16) *	73 (16) *	71 (17) *	73 (18) *	84 (12) *
BMI (kg/m ²)	27.2 (7.1) *	27.6 (6.6) *	26.6 (5.5) *	30.0 (6.5) *	28.2 (7.8) *
Haemoglobin (g/dL)	10.1 (1.9) *	9.6 (1.8) *	9.7 (2.0) *	9.2 (1.5) *	9.7 (1.8) *
Platelet count (x10 ⁹ /L)	240.5 (42,779) **	241 (8, 728) **	243 (21,592) **	226.5 (41,587) **	219 (163,419) **
Neutrophil count (x10 ⁹ /L)	5.1 (1.0, 26.6) **	7.1 (0.8,30.6) **	7.1 (1.5,31.7) **	8.6 (3.9,18.5) **	5.9 (3.8,11.1) **
Lymphocyte count (x10 ⁹ /L)	1.4 (0.3,3.9) **	1.1 (0.3,19.7) **	1 (0.2, 21.1) **	1.2 (0.4,17.6) **	1.3 (0.4,2.7) **
C-reactive protein (mg/L)	27 (1,297) **	53 (1,487) **	64 (2,375) **	91 (3,282) **	23 (11,402) **
Albumin (g/L)	29 (13,43) **	26.5 (10,45) **	27 (12,43) **	25.5 (11,38) **	29 (11,39) **
Calcium (Adjusted) (mmol/L)	2.42 (0.18) *	2.31 (0.21) *	2.32 (0.24) *	2.26 (0.24) *	2.38 (0.16) *
Phosphate (mmol/L)	1.64 (0.56) *	1.83 (0.75) *	1.95 (0.73) *	1.67 (0.59) *	1.93 (0.61) *
Calcium phosphate product	3.98 (1.43) *	4.20 (1.77) *	4.49 (1.68) *	3.76 (1.35) *	4.58 (1.50) *

Table 3.1 – Clinical and laboratory characteristics from the date of catheter insertion for each central venous catheter sub-type. Data values are expressed as value (%), *mean (SD) or **median (min, max). ‘TCVC’ = tunnelled central venous catheter. ‘NTCVC’ = non-tunnelled central venous catheter. ‘IJug’ = internal jugular vein insertion site. ‘Fem’ = femoral vein insertion site. ‘(G)’ = catheter exchange procedure conducted over a guidewire. ‘CRB’ = catheter-related bacteraemia. ‘ARF’ = acute renal failure. ‘A/CRF’ = acute on chronic renal failure. ‘CRF’ = chronic renal failure. ‘RRT’ = renal replacement therapy. ‘Anticoagulation’ = warfarin or antiplatelet use. ‘SBP’ = systolic blood pressure. ‘DBP’ = diastolic blood pressure. ‘BMI’ = body mass index.

In total, 301/823 (36.6%) of procedures were insertions of tunnelled central venous catheters (TCVC) whilst 522/823 (63.4%) were insertions of non-tunnelled central venous catheters (NTCVC). Of the NTCVC insertions, 373/522 (71.5%) were inserted into the internal jugular veins with 149/522 (28.5%) inserted into the femoral veins. 73/522 (14.0%) of NTCVC insertions were conducted as catheter-exchange procedures over a guidewire. A total of 44,528 catheter days were accumulated over the study period during which time there were 115 cases of catheter-related bacteraemia (2.57 per 1000 catheter days) and 131 cases of catheter removal due to poor haemodialysis blood flow (2.94 per 1000 catheter days).

Bacteraemia

115 cases of catheter-related bacteraemia occurred with 122 bacterial isolates identified on blood culture. Staphylococcal sub-species accounted for the majority of cases with 47/122 (38.5%) isolates of staphylococcus epidermidis, 29/122 (23.8%) isolates of methicillin-sensitive staphylococcus aureus and 9/122 (7.4%) isolates of methicillin-resistant staphylococcus aureus. Gram negative bacilli accounted for 19/122 (15.6%) isolates, other gram positive cocci in 14/122 (11.5%) bacterial isolates and gram positive bacilli in 4/122 (3.3%) isolates.

Rates of catheter-related bacteraemia were 1.77 per 1000 catheter days in the TCVC group, 6.3 per 1000 catheter days in the internal jugular vein NTCVC group and 13.5 per 1000 catheter days in the femoral vein NTCVC group. Internal Jugular and femoral NTCVCs exchanged over a guidewire demonstrated catheter-related bacteraemia rates of 9.7 and

21.5 per 1000 catheter days respectively. Events occurred at median (range) of 54 (28, 127) days in the TCVC group, 10 (5, 18) days in the internal jugular NTCVC group, 4 (4, 5) days in the internal jugular guidewire exchange group, 6 (1, 8) days in the femoral NTCVC group and 8 days in the femoral guidewire exchange group.

Using the TCVC group as a comparator, all types of NTCVC insertion procedure were significantly associated with catheter-related bacteraemia on univariate analysis ($p<0.001$). Other variables significantly associated with the subsequent development of catheter-related bacteraemia included higher haemodialysis blood flow (265mls/min v 250mls/min, $p=0.002$) and shorter durations of catheter lifespan (50 days v 83 days, $p<0.001$). This, however, reflects our unitary practice of removing catheters once a diagnosis of catheter-related bacteraemia has been made unless there is a strong clinical reason to try and salvage the catheter. Trends towards a significant association with catheter-related bacteraemia on univariate testing included higher serum adjusted calcium levels ($p=0.004$), an elevated Modified Charlson comorbidity score ($p=0.005$), being on antibiotic at the time of catheter insertion ($p=0.008$), a diagnosis of diabetes ($p=0.011$) and a longer duration on renal replacement therapy ($p=0.013$). All other variables were not significantly associated with outcome on univariate analysis.

Multivariate analysis demonstrated hazard ratios (HR) for the development of bacteraemia in patients dialysing with internal jugular NTCVCs of 2.93 ($p<0.001$), femoral NTCVCs of 5.97 ($p<0.001$), 6.42 ($p<0.001$) for internal jugular NTCVCs exchanged over a guidewire and 9.84 ($p=0.002$) for femoral NTCVCs exchanged over a guidewire (figure 3.1). There was also a significant independent association between an elevated modified Charlson

comorbidity score with a HR of 1.102 ($p=0.034$). All other variables failed to reach statistical significance on multivariate testing (table 3.2).

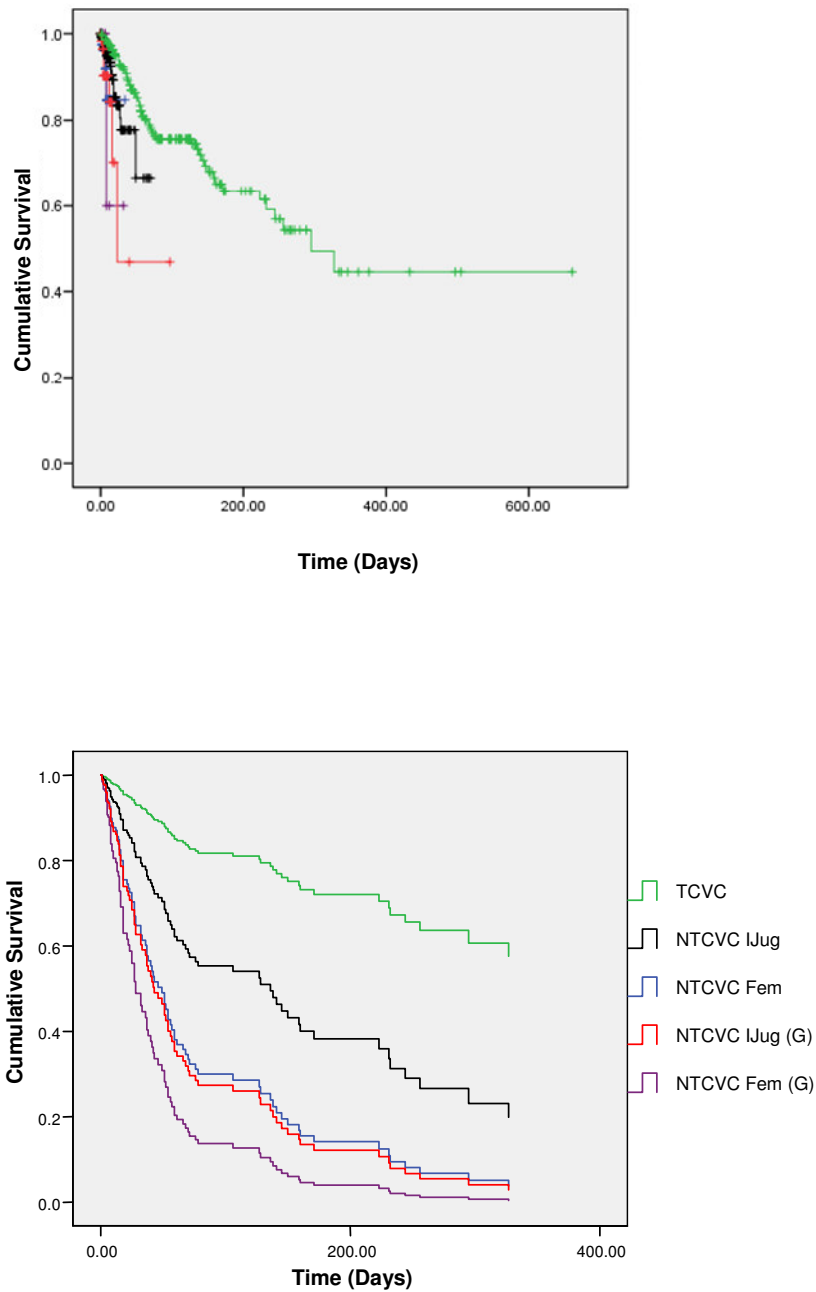


Figure 3.1 – Univariate Kaplan-Meier and Multivariate analysis adjusted catheter-related bacteraemia free survival plotted for each central venous catheter sub-type.

Variable	Univariate Analysis (p-value)	Multivariate Analysis (Hazard Ratio)	P-value
NTCVC Femoral (Guidewire)	p<0.001	9.84	p=0.002
NTCVC Internal Jugular (Guidewire)	p<0.001	6.42	p<0.001
NTCVC Femoral	p<0.001	5.97	p<0.001
NTCVC Internal Jugular	p<0.001	2.93	p<0.001
Modified Charlson Comorbidity Score	p=0.005	1.102	p=0.034
Haemodialysis Blood Flow (mls/min)	p=0.002	-	p=NS

Table 3.2 – Variables entered into multivariate analysis and the associated risk of subsequent catheter-related bacteraemia. (Guidewire) refers to catheter exchange procedures over a guidewire.

Poor Flow

Rates of catheter removal due to poor flow were 0.98 per 1000 catheter days in the TCVC group, 12.3 per 1000 catheter days in the internal jugular vein NTCVC group, and 37.6 per 1000 catheter days in the femoral vein NTCVC group. Internal Jugular and femoral NTCVCs exchanged over a guidewire demonstrated failure rates of 20.2 and 32.3 per 1000 catheter days respectively.

Events occurred at median (range) of 57.5 (6, 337) days in the TCVC group, 6 (0, 22) days in the internal jugular NTCVC group, 5 (1, 10) days in the internal jugular guidewire exchange group, 3 (1, 10) days in the femoral NTCVC group and 1 (0, 2) days in the femoral guidewire exchange group.

Using the TCVC group as a reference for comparison, all types of NTCVC insertion procedure were significantly associated with catheter removal due to poor flow on univariate analysis ($p<0.001$). Other variables significantly associated with catheter removal due to poor haemodialysis blood flow were low haemodialysis blood flow during the first dialysis following catheter insertion (237mls/min v 255mls/min, $p<0.001$) and elevated levels of CRP at the time of catheter insertion (61mg/dL v 40mg/dL, $p<0.001$). Patients who required catheter replacement due to poor flow were more likely to have their catheter removed earlier (36 days v 58 days, $p<0.001$).

Trends towards a significant association with poor flow were seen with high platelet counts ($p=0.067$) and lower levels of serum albumin ($p=0.087$). All other variables were not associated with outcome on univariate analysis.

Multivariate analysis demonstrated hazard ratios (HR) for the removal of catheters due to poor haemodialysis blood flow in patients dialysing with internal jugular NTCVCs of 4.65 ($p<0.001$), femoral NTCVCs of 9.23 ($p<0.001$), 5.56 ($p<0.001$) for internal jugular NTCVCs exchanged over a guidewire and 11.73 ($p<0.001$) for femoral NTCVCs exchanged over a guidewire (figure 3.2).

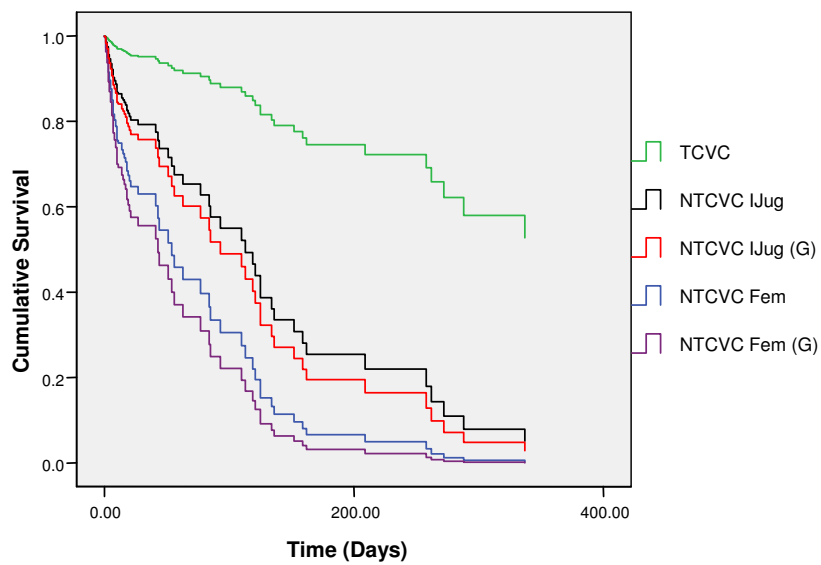
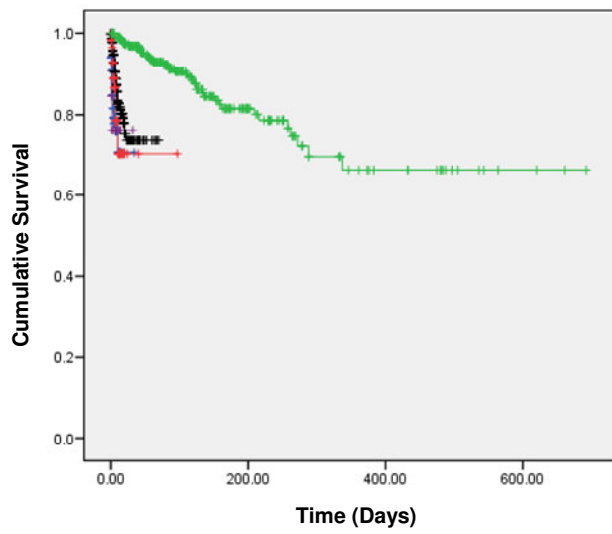


Figure 3.2 – Univariate Kaplan-Meier and Multivariate analysis adjusted catheter survival with regard episodes of catheter removal due to insufficient haemodialysis blood flow. Survival plotted for each central venous catheter sub-type.

There was also a significant independent association between an elevated CRP with a HR of 1.004 ($p<0.001$) per unit increase in CRP and HR=0.992 with haemodialysis blood flow immediately following catheterisation ($p<0.001$) to subsequent removal of catheter due to poor flow (table 3.3).

Variable	Univariate Analysis (p-value)	Multivariate Analysis (Hazard Ratio)	P-value
NTCVC Femoral (Guidewire)	$p<0.001$	11.73	$p<0.001$
NTCVC Femoral	$p<0.001$	9.23	$p<0.001$
NTCVC Internal Jugular (Guidewire)	$p<0.001$	5.26	$p<0.001$
NTCVC Internal Jugular	$p<0.001$	4.65	$p<0.001$
C-reactive Protein (mg/dL)	$p=0.002$	1.004	$p<0.001$
Haemodialysis Blood Flow (mls/min)	$p=0.002$	0.992	$p<0.001$

Table 3.3 – Variables entered into multivariate analysis and the associated risk of subsequent catheter removal due to poor haemodialysis blood flow. (Guidewire) refers to catheter exchange procedures over a guidewire

3.4 Discussion

These data demonstrate the significant differences between tunnelled and non-tunnelled central venous haemodialysis catheter insertion procedures in terms of rates of catheter-related bacteraemia and catheter failure due to poor haemodialysis blood flow. Importantly, they bring clarity to the debate of whether such adverse events develop as a

function of the characteristics of the catheterisation procedure in terms of tunnelling and site of insertion, or the characteristics of the patient receiving the catheter.

From these data, it can be concluded that the characteristics of the catheterisation procedure in terms of tunnelling and site of insertion, had the greatest independent effect on risk of developing both catheter-related bacteraemia and catheter failure due to poor haemodialysis blood flow of all the variables studied. The clear hierarchy of complication rates across different types of catheter insertion procedure were demonstrated as being independent of established measures of comorbidity in haemodialysis patients, including haemoglobin, bone biochemistry, dialysis dose, markers of inflammatory burden, in the setting of acute renal failure, diabetes and modified Charlson comorbidity index score (103-105).

Of the variables studied, the only other factor independently associated with catheter-related bacteraemia was an elevated modified Charlson comorbidity index score at the time of catheter insertion. The only other factor independently associated with catheter failure due to poor haemodialysis blood flow was elevated CRP at the time of catheter insertion.

The wide range of clinical and laboratory variables included in the analysis allowed many of the potential confounders to be taken into account. Not all potential confounding variables were, however, included. The number and type of vascular access procedures each patient had undergone prior to catheterisation was one variable that was not reliably recorded in the EPR and as such was not recorded during data collection. It can be envisaged that patients with poor peripheral access or central venous stenosis may have a

higher turnover catheter insertions and thus may gradually depend more heavily on NTCVCs, use of insertion sites such as the femoral veins and/or use of guidewire catheter-exchange procedures. Such patients could account for the especially high hazard ratios found with these procedures. Whilst the large number of procedures, long period of follow-up and inclusion of variables such as the patient's duration on RRT may partly adjust for this issue in study design, this study does not sufficiently address this potentially important risk factor. Closer scrutiny of the complex vascular access patient could be achieved by future studies adjusting for variables such as the number of previous vascular access procedures, and reporting subsequent rates of conversion onto a functioning AVF or graft and subsequent sustainability of that AVF or graft.

The use of a clinical definition of CRB may also be regarded as a limitation. The most formal definition of CRB requires concurrent line and peripheral blood cultures, both growing the same organism and with either 5 times as many colony forming units in the catheter culture or positive growth at least 2 hours before the peripheral culture. For these criteria to be met requires the availability of dialysis unit nursing staff who are trained in peripheral venepuncture, patients with adequate peripheral veins to allow venepuncture to take place or in whom preservation of the peripheral veins for future arteriovenous fistula creation is not required, and suitable culture storage and analysis facilities on site. Such requirements are a logistical hurdle in studies of simple observational design and thus may result in underreporting of clinically significant bacteraemia. It was for these reasons that a clinical definition of CRB was used, albeit at the risk of over-reporting the rate of true bacteraemia arising from the catheter.

Another important limitation concerns the data on guidewire catheter-exchange procedures. The analysis was limited by not adjusting for the period of time spent with a catheter prior to it being exchanged via a guidewire. In this regard, we cannot exclude that the higher event rates seen in this group do not simply reflect the effect of increased time with a catheter in-situ. Whilst increased catheter lifespan was associated with a lower rate of CRB, this simply reflected the local unitary practice of removing a catheter once CRB had been diagnosed and as such does not help clarify this issue.

How does catheter insertion site contribute to bacteraemia risk? The pattern of these results suggest that catheters inserted into a site which has been previously used (i.e. a previous catheter tunnel used with a catheter exchange procedure over a guidewire) or in skin folds such as the femoral creases may herald locations where pathogens are more likely to reside. How does catheter tunnelling relate to bacteraemia risk? Our data show that catheters entering the vein close to the skin surface without a period of subcutaneous tunnelling are more likely to become complicated by infection and/or thrombosis. The shorter physical distance between skin surface and vein, and thus a shorter length of exposure to innate immune defences may go some way to explain the better bacteraemia rates seen with subcutaneous tunnelling of the catheter. With regard to the differences between catheter types due to poor haemodialysis flow, the tunnelled catheters used in this cohort were of greater diameter than their non-tunnelled counterparts and thus may account for the better thrombosis rates seen in this group. Within the NTCVC groups, the main difference in flow rates was between catheters inserted into the internal jugular veins and the femoral veins. This may simply reflect the different calibre, tortuosity and extrinsic compression of the venous tree in these locations. Further study of these theories would be of benefit.

Several organisations have drawn up clinical guidelines to address the issue of optimal catheter practice. The UK Renal Association Guidelines, for instance, suggest that less than 20% of patients on long-term haemodialysis should use tunnelled or non-tunnelled central venous catheters as their mode of vascular access (48). NKF-KDOQI cite the lower complication rate associated with tunnelled central venous catheters compared with non-tunnelled catheters in their recommendation that tunnelled catheters are used where the period of catheter use is likely to be longer than 3 weeks. In cases of suspected catheter thrombosis or low-grade catheter-related bacteraemia in the absence of subcutaneous tunnel infection, NKF-KDOQI suggests that the catheter may be exchanged over a guidewire (47). Our data suggest that future guidelines should give greater emphasis on early tunnelled central venous catheterisation where possible, minimising non-tunnelled central venous catheter use and limiting guidewire catheter-exchange to cases where re-catheterisation at a separate site is unachievable.

3.5 Conclusion

In conclusion, our data suggest that tunnelled central venous catheter insertions are associated with lower complication rates than non-tunnelled central venous catheter insertions, independent of whether patients have acute or chronic renal failure, or high levels of comorbidity. When considering central venous catheterisation, these data suggest that tunnelled catheter insertion be sought when patients have no pre-existing functioning arteriovenous fistula or graft. In cases where tunnelled central venous catheterisation

cannot be performed, non-tunnelled catheterisation of the internal jugular veins should be sought. Insertion of a non-tunnelled femoral venous catheter is associated with the next highest rates of bacteraemia and failure due to poor haemodialysis blood flow and therefore should be used in selected cases only. The practice of catheter-exchange over a guidewire should be restricted to cases where primary catheterisation at a de novo site cannot be achieved.

Chapter 4

A Prospective Randomised Controlled Trial Comparing the Effect of Heparinised Catheter Lock Solutions on Systemic Anticoagulation in Haemodialysis Patients

4.1 Background

Haemodialysis catheter thrombosis is a common problem. Thrombus formation on the catheter surface occurs within a matter of hours (106) and this may extend to involve thrombosis of the central veins the longer the catheter remains in-situ (107). A clear association between thrombus formation and subsequent catheter-related bacteraemia has been demonstrated (108).

Presently there are no published NKF-DOQI guidelines on evidence-based practice regarding the prevention of catheter thrombosis. The majority of renal units simply rely on using heparin as a catheter-locking solution, where filling the internal lumens of the catheter with heparin solutions prevents the build up of thrombus. Evidence to date suggests that heparin lock solutions significantly decrease catheter thrombosis, decrease bacterial colonisation of the catheter and may decrease catheter-related bacteraemia (109).

The benefits of maintaining catheter patency with heparin-locking are, however, balanced against the risk of systemic heparinisation and subsequent bleeding. Haemodialysis patients are already predisposed to bleeding because of uraemia or associated coagulopathy. Several renal units in the UK have observed major haemorrhagic events requiring blood transfusion following central venous catheter locking with heparin. The balance of risk versus benefit is, however, uncertain and thus heparin-locking practice is found to vary widely among renal units (110, 111). Several studies have been performed to demonstrate the risks/benefits of different heparin locking practices however the majority of studies have been observational, non-blinded and retrospective in design (56).

In this study an investigator-blinded randomised controlled trial of heparin catheter lock solutions in non-tunnelled (temporary) central venous catheters was undertaken. The working hypothesis was that higher concentrations of heparin solution would lead to significantly greater disturbance of systemic coagulation as measured by the change in activated partial thromboplastin time (APTT) at 10 minutes post catheter locking. The study was designed with a primary endpoint of change in APTT at 10 minutes following catheter locking with heparin 1,000U/ml and 5,000U/ml. Secondary study endpoints included subsequent catheter lifespan, catheter thrombosis rates and rates of catheter-related bacteraemia between groups.

4.2 Methods

Full approval was granted for this study from our local research and ethics committee. 34 consecutive patients requiring insertion of a temporary non-tunnelled dual lumen central venous haemodialysis catheter were approached of which 28 patients gave informed consent and were recruited into the study. Patients were either starting haemodialysis for the first time or were established on renal replacement therapy (RRT) and required temporary central venous catheterisation because of vascular access failure. Patients with exposure to heparin in the previous 24 hours were excluded from the study and thus were not asked to take part. On entry to the study, patients underwent third party randomisation to either heparin 5000iu/mL or heparin 1000iu/mL as catheter lock solution. All members of the investigating team were blind to the allocation of heparin solution.

Standard unitary protocol for catheter care was employed throughout the observation period. Specifically, this demanded complete sterile barrier precautions during catheter

insertion and when manipulating the catheter hub. Following catheter-hub manipulation, the skin surrounding the insertion site was soaked with chlorhexidine solution prior to a sterile dressing being applied.

Once the catheter was inserted and sutured in-situ, a 5ml blood sample was taken from the catheter for measurement of the APTT. Each lumen of the catheter was then flushed with 20ml 0.9% Saline using a positive pressure technique to remove any blood from the lumen. Both lumens of the catheter were then slowly filled with a volume of the allocated heparin solution equal to the volume of each lumen as stated by the manufacturer. A stop-clock was then started and at 10 minutes following catheter locking a peripheral venous blood sample taken for repeat measurement of the APTT. The same concentration of heparin was used to lock the catheter after each successive dialysis session until the catheter was removed.

At the time of catheter insertion baseline clinical, demographic and laboratory variables for each patient were retrieved from the unitary electronic patient records and clinical case records. Where multiple measurements of a single variable had been recorded, the most recent value was used. Clinical variables collected were age, gender, primary renal diagnosis, the presence or absence of diabetes, body mass index (BMI), systolic and diastolic blood pressure, anticoagulant use, statin use, immunosuppressant medication, length of time on renal replacement therapy, and vascular access flow rate on haemodialysis. Laboratory variables collected at study entry were haemoglobin, platelet count, neutrophil count, lymphocyte count, serum C-reactive protein (CRP), serum albumin, serum adjusted calcium and phosphate product.

Outcomes

Each patient was followed up for the period over which the catheter remained in-situ. The primary outcome was the difference in APTT at 10 minutes following catheter locking between heparin 5000iu/mL and heparin 1000iu/mL. Secondary outcomes included cases of catheter removal due to insufficient haemodialysis blood flow to maintain haemodialysis and cases of catheter-related bacteraemia (CRB).

CRB events were determined by analysis of all positive blood culture results from the renal unit reported by the bacteriology laboratory during the period of study in conjunction with analysis of the patient's clinical notes and electronic patient record. Bacteraemia was deemed significant if positive blood cultures were associated with a raised systemic inflammatory response (e.g. pyrexia, raised CRP, raised white-cell count). This approach is in keeping with the consensus definition of clinically significant bacteraemia and consistent with that used in routine clinical practice.

Catheter removal due to poor flow was defined as removal of a central venous catheter in response to low haemodialysis blood flows that consistently impaired effective haemodialysis delivery despite optimal anticoagulation and/or thrombolytic intervention. This reflected routine clinical practice in the participating renal unit. All decisions regarding catheter removal were made by the clinical team responsible for each patient's care and were made independently from the investigator.

Analysis & Study Protocol

Statistical analysis was performed by SPSSTM version 14.0 (SPSS Inc, IL, USA). Normality testing was performed on all of the recorded continuous variables. Student's t-testing and Mann-Whitney U testing were then used to assess differences between groups where appropriate. The primary outcome sought was the difference in the APTT ratio between groups after administration of the heparin lock solutions following catheter insertion as determined by student's t-testing. Bacteraemia-free survival and catheter failure secondary to poor haemodialysis blood flow survival rates for each group was assessed by Kaplan-Meier survival analysis and Log Rank testing with $p < 0.05$ being regarded as statistically significant. A sample size of 12 participants in each group was determined as providing 80% power to detect a 25% difference in mean APTT from 28s to 35s with standard deviation of 6s and significance level of $p < 0.05$. Randomisation was undertaken independently by the clinical trials pharmacy unit at Glasgow Royal Infirmary. Block randomisation was used with the aim to recruit greater numbers than those determined by the power calculation in an effort to pursue suitable numbers of secondary outcome events.

All aspects of the clinical trial were conducted in accordance with national guidance from the UK Medical Research Council and the Department of Health. Trial sponsorship was undertaken by NHS Greater Glasgow as the employer of the chief investigator, Dr Robert Mactier. The Research and Development department of Glasgow Royal Infirmary were consulted where permission was sought and granted for the study to be conducted within NHS Greater Glasgow. The study protocol was further developed by the Glasgow Royal Infirmary clinical trials pharmacy unit. During this time consultation was made with the

Medicines and Healthcare products Regulatory Agency where it was determined that studies of catheter lock solutions are regarded as studies of medical devices and thus MHRA approval was not required. Following peer review the study protocol was submitted to the local research ethics committee where permission for the study to proceed was granted. The study protocol is detailed in figure 4.1 below.

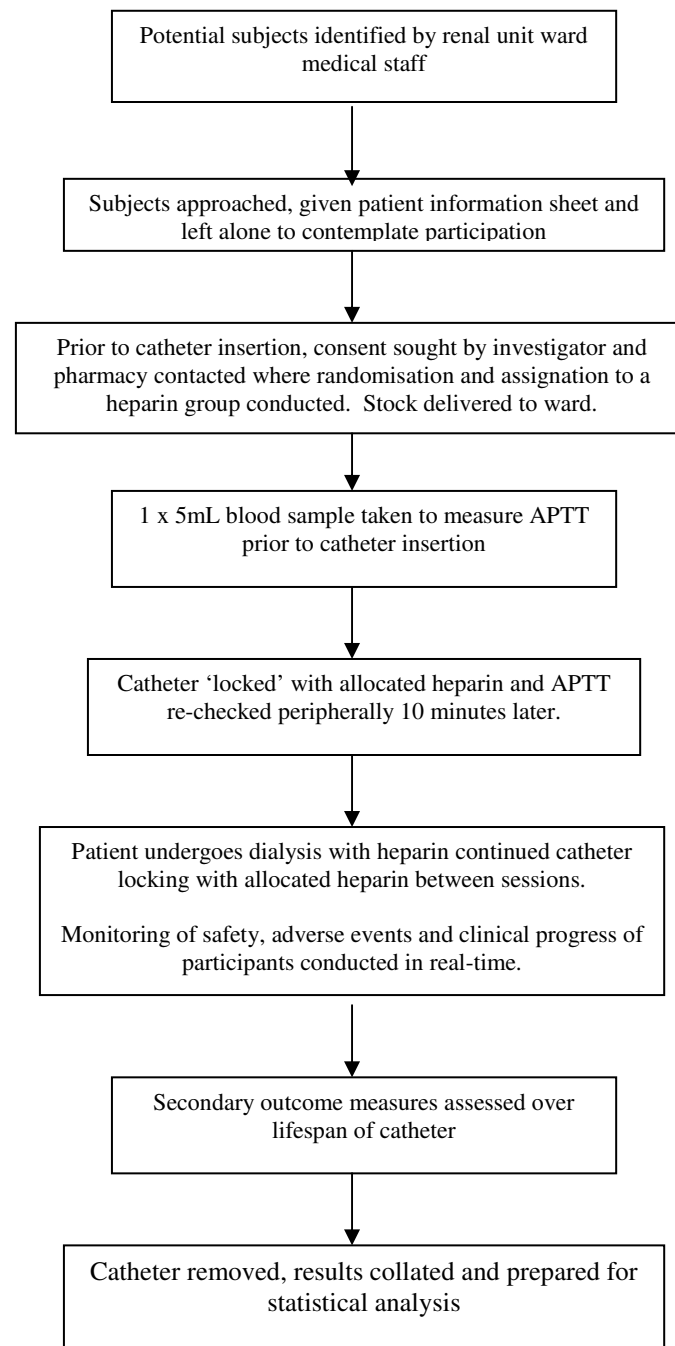


Figure 4.1 – Flow chart detailing the study design from recruitment to collation of results.

4.3 Results

28 patients who met the inclusion criteria provided informed consent to participate in the study. 16/28 (57.1%) patients were male. Median age of the cohort was 68.0 years (range 40.2 – 84.8).

15/28 (53.6%) presented with acute renal failure, defined as a duration of renal replacement therapy (RRT) of less than 90 days with no known pre-existing renal disease. 7/28 (25.0%) presented with acute on chronic renal failure, defined as a duration of RRT less than 90 days with known pre-existent renal disease. 6/28 (21.4%) patients had established renal failure with duration on RRT of median 737.5 days (range 329 - 7505 days).

13 patients were randomised to the heparin 1000iu/mL group whilst 15 patients were randomised to the heparin 5000iu/mL group. Details of all clinical and laboratory variables recorded on the date of study entry for each group are listed in table 4.1 below. No statistically significant differences were demonstrated between the heparin 1000iu/mL group and the heparin 5000iu/mL group on univariate testing although trends towards a positive association were found when considering the proportion of patients known to have a diagnosis of diabetes with a greater proportion of such patients being demonstrated in the heparin 5000iu/mL group.

Variable	Heparin 1000iu/mL	Heparin 5000iu/mL	P-value
No. of Patients	N=13	N=15	-
Male:Female	7:6	9:6	p=0.99
ARF (N)	7/13	8/15	p=0.99
A/CRF (N)	5/13	2/15	p=0.20
CRF (N)	1/13	5/15	p=0.17
Diabetes (N)	4/13	11/15	p=0.06
Statin (N)	6/13	8/15	p=0.99
Aspirin (N)	5/13	7/15	p=0.72
Clopidogrel (N)	0/13	2/15	p=0.48
Immunosuppression (N)	2/13	1/15	p=0.58
Duration on RRT (days)	0 (0, 460) **	9 (0, 7505) **	p=0.19
SBP (mmHg)	130 (24) *	133 (26) *	p=0.78
DBP (mmHg)	72 (22) *	74 (13) *	p=0.86
BMI (kg/m ²)	34.6 (6.7) *	28.3 (5.2) *	p=0.07
Haemoglobin (g/dL)	9.2 (1.2) *	9.2 (1.6) *	p=0.99
Platelet count (x10 ⁹ /L)	214 (37, 366) **	272 (73, 623) **	p=0.30
Neutrophil count (x10 ⁹ /L)	8.5 (4.4) *	8.4 (4.9) *	p=0.95
Lymphocyte count (x10 ⁹ /L)	1.2 (0.5) *	1.4 (0.7) *	p=0.35
C-reactive protein (mg/L)	52 (3, 220) **	29 (3, 74) **	p=0.17
Albumin (g/L)	23 (14, 37) **	29 (15, 42) **	p=0.27
Calcium (Adjusted) (mmol/L)	2.36 (0.17) *	2.29 (0.18) *	p=0.29
Phosphate (mmol/L)	1.48 (0.55) *	1.84 (0.62) *	p=0.11

Table 4.1 – Clinical and laboratory characteristics of heparin 1000iu/mL and heparin 5000iu/mL groups. Data values are expressed as value (%), *mean (SD) or **median (min, max). ‘ARF’ = acute renal failure. ‘A/CRF’ = acute on chronic renal failure. ‘CRF’ = chronic renal failure. ‘RRT’ = renal replacement therapy. ‘SBP’ = systolic blood pressure. ‘DBP’ = diastolic blood pressure. ‘BMI’ = body mass index. Values recorded as mean (SD) or median (minimum, maximum).

The measured baseline APTT immediately prior to catheter heparin-locking did not significantly differ between groups with mean (SD) APTT of 28 (2.9) in the heparin 1000iu/mL group compared to mean (SD) APTT of 27.7 (5.9) in the heparin 5000iu/mL group ($p=0.88$). The measured APTT at 10 minutes post catheter heparin-locking was significantly different between groups with mean APTT of 40.8 in the heparin 1000iu/mL group compared with mean APTT of 129.7 in the heparin 5000iu/mL group ($p<0.001$). When expressed as the percentage rise in APTT from baseline to 10 minutes, there was a statistically significant difference between groups of median +22.2% (range 0, 210) rise in APTT in the heparin 1000iu/mL group compared with +373.7% (range 133, 800) in the heparin 5000iu/mL group ($p<0.001$). This is demonstrated in table 4.2.

Average catheter lifespan was 7 days in both heparin 1000iu/mL and heparin 5000iu/mL groups ($p=0.90$). Catheter failure secondary to insufficient haemodialysis blood flow occurred in 3/13 cases in the heparin 1000iu/mL group compared with 4/15 cases in the heparin 5000iu/mL group ($p=0.88$). The rates of catheter-related bacteraemia were similar with 2/13 cases in the heparin 1000iu/mL group compared with 2/15 cases in the heparin 5000iu/mL group ($p=0.83$). This is demonstrated in table 4.2.

Recorded mean haemodialysis blood flow for the lifespan of the catheter was similar in both groups at 235mls/min in the heparin 1000iu/mL group compared with 231mls/min in the heparin 5000iu/mL group ($p=0.77$). Median c-reactive protein measured over the lifespan of the catheter was similar in both groups at 51mg/L in the heparin 1000iu/mL group versus 47mg/L in the heparin 5000iu/mL group ($p=0.39$). This is demonstrated in table 4.2.

Variable	Heparin 1000iu/mL	Heparin 5000iu/mL	P-value
APTT at 0mins (seconds)	28 (2.9) *	27.7 (5.9) *	p=0.88
APTT at 10mins (seconds)	40.8 (18.6) *	129.7 (47) *	p<0.001
Change in APTT at 10mins (%)	+22.1% (0, 210) **	+373.8% (133, 800) **	p<0.001
Catheter Lifespan (days)	7	7	p=0.90
Catheter-Related Bacteraemia	N=2/13	N=2/15	p=0.88
Catheter Thrombosis	N=3/13	N=4/15	p=0.83
Haemodialysis blood flow (mls/min)	235 (26) *	231 (30) *	p=0.77
Mean c-reactive protein (mg/L)	51 (6, 313) **	47 (11, 91) **	p=0.39

Table 4.2 – Comparison of outcomes between heparin 1000iu/mL and heparin 5000iu/mL groups. ‘APTT’ = activated partial thromboplastin time. Values recorded as *mean (SD) or **median (minimum, maximum).

4.4 Discussion

This study has demonstrated that use of heparin as a catheter-locking agent in haemodialysis patients is associated with a significant degree of systemic heparinisation as demonstrated by prolongation of the activated partial thromboplastin time (APTT). Systemic heparinisation occurred in both groups despite only instilling a volume of heparin equal to the internal catheter luminal volume stated by the catheter manufacturer. It is therefore likely that despite this method of heparin instillation, overspill of the injected heparin occurs. As a result, patients in both groups were exposed to an increased risk of haemorrhage in the hours immediately following catheter insertion and after catheter-locking following each haemodialysis treatment. There were, however, no instances of

haemorrhage in either group over the course of the study, albeit within a relatively small cohort with a relatively short duration of follow-up.

We found that the use of heparin 1000iu/mL conferred a significantly lower risk of systemic heparinisation than heparin 5000iu/mL. These findings were demonstrated in the setting of a prospective, investigator-blinded, randomised study and confirm similar findings reported in non-randomised non-blinded observational studies (110, 111). The lack of any significant difference between groups with regard to the secondary outcome measures of catheter-related bacteraemia and catheter failure due to poor haemodialysis blood flow is of limited value due to the small sample size, the lack of power to examine these outcomes, the heterogeneity of non-tunnelled catheter insertion sites and the relatively short duration of follow-up. These limitations must also be considered when considering the primary outcome measure. The mixture of acute and chronic renal failure patients, the presence of diabetes, concurrent comorbid conditions and anticoagulant and antiplatelet use could be accounted for more comprehensively by greater numbers of study participants.

These findings support those suggested by previous retrospective observational studies. The routine use of a 5,000U/ml heparin lock solution equal to the estimated catheter lumen volumes in 13 haemodialysis patients in an ICU setting in Scotland was associated with an APTT ratio >7 in 53% of the 32 paired samples available for analysis from these patients (111). Several studies have observed good catheter patency rates when 1000iu/mL heparin lock solutions were used in the control group of prospective studies designed to examine the effect of antibiotic lock techniques on the incidence of catheter-related bacteraemia (112, 113). Whether use of 1,000iu/mL heparin is a suitable alternative across a large

population of haemodialysis patients is uncertain as there is some evidence uncovered in larger populations that catheter patency may be compromised. In a Canadian longitudinal study of 6940 haemodialysis sessions the use of a 1000iu/mL heparin lock solution in central venous haemodialysis catheters was not associated with higher rates of catheter malfunction compared with the use of 10,000iu/mL heparin solution but the 1000iu/mL group did require greater anti-thrombotic intervention with thrombolytic drugs (56).

4.5 Conclusion

The optimal method of heparin-locking dialysis catheters remains uncertain. Balancing the risks of catheter-thrombosis and subsequent catheter failure due to under-anticoagulation against the risk of bleeding and haemorrhage associated with increasingly concentrated heparin catheter lock solutions is of primary importance. By demonstrating the significantly higher risk of systemic heparinisation with heparin 5000iu/mL compared to heparin 1000iu/mL, this randomised, investigator-blinded controlled trial helps quantify the contribution heparin concentration makes to one side of this debate, that of increased risk of bleeding. This work also demonstrates the need for a large randomised controlled trial to determine the optimal concentration of heparin solution with regard to risk of catheter-thrombosis, catheter-related bacteraemia and significant haemorrhage.

Chapter 5

An In-Vitro Analysis of Vancomycin in Combination with Heparin as an Antimicrobial Haemodialysis Catheter Lock

Solution

5.1 Background

The work detailed in chapters 2 and 3 demonstrate staphylococcal sub-species to be the most prevalent of pathogens in haemodialysis patients with bacteraemia. The high prevalence of *Staphylococcus epidermidis* and *Staphylococcus aureus* bacteraemia in patients with catheter-related infection has been demonstrated in similar studies of patients with catheter related infection (114). These bacteria are endemic on the skin surface and constitute normal skin flora. If, however, a patient has an indwelling medical device such as a catheter this may become colonised by these flora. Initially a catheter may be colonised by only one single organism, for example, *S. epidermidis*, however, the longer a catheter remains in place, more diverse microbial communities may form and involve other organisms such as *klebsiella pneumoniae* and *proteus mirabilis* (115). Many of these organisms have the ability to form biofilm which has been found to be the underlying substrate to catheter infection, bacteraemia and resultant systemic infection (116, 117).

Biofilms were first identified in 1943 by Zobell however it was not until the 1970s that their role in causing infection was realised (118). Many definitions of biofilms have evolved over the years as understanding and knowledge of biofilm structures has developed. A biofilm is said to be a complex community of sessile cells that attach to a substratum and to each other. They are embedded within a matrix of self-producing polymeric substances and they have altered phenotypes due to differences in growth rates and gene transcription in comparison to their planktonic counterparts (115). Some bacteria, such as certain types of staphylococci produce extracellular slime which may interfere with the phagocytic activity of macrophages and thus make bacteria within the biofilm more resistant to innate immune responses such as killing by active oxygen species in

leukocytes. These mechanisms allow biofilms to develop resistance to biocides, phagocytes and antimicrobials with the degree of resistance correlating with the maturity of the biofilm (119-121). It is well recognised that successful treatment of CRB requires the successful eradication of biofilm (119).

The antibiotic lock technique has been developed as a method of preventing and eradicating biofilm on the endoluminal surface of haemodialysis catheters. This involves instillation of a concentrated antibiotic-anticoagulant into the catheter lumen that is left to dwell between haemodialysis sessions. This method delivers a small absolute amount of antibiotic to the patient but achieves a high local concentration in the catheter lumen that is 100 to 5,000 times higher than the minimum inhibitory concentration (MIC) for the infecting bacterium. Use of antibiotic lock solutions as a primary prevention strategy has raised concerns regarding the risks of developing antimicrobial resistance with leakage of the antibiotic-lock solution into the circulation. Using antibiotic-lock solution as a secondary adjunctive measure to systemic antibiotics in treating catheter-related bacteraemia (CRB) is, however, less contentious.

The antibiotic-lock technique requires the safe and efficacious combination of an antibiotic and an anticoagulant. Heparin is a well-established anticoagulant used for locking haemodialysis catheters. It is stable in combination with antimicrobials (122) although does not demonstrate anti-microbial activity itself (123). Several studies have used heparin in conjunction with various antibiotics and demonstrated stability in combination and positive in-vitro and in-vivo benefits in preventing and treating infection over the short term (122, 124, 125). Whilst this area continues to evolve, our understanding of how

combinations of anticoagulant and antimicrobial interact is limited. In this study the effect of heparin on the bactericidal activity of the glycopeptide vancomycin on free floating planktonic and biofilm-associated staphylococci was examined. The work was conducted with a null hypothesis of heparin having no significant effect on the ability of staphylococci to interact with vancomycin.

5.2 Methods

Bacterial Strains

Six clinical isolates of *S. aureus*, obtained from the Scottish MRSA Reference Laboratory (SMRSARL) (Stobhill Hospital, Glasgow, UK), were selected on the basis of their recognised capacity to form biofilm. These isolates had been genotyped at the SMRSARL using either pulsed-field gel electrophoresis (PFGE) for methicillin resistance *S. Aureus* (MRSA) isolates using the Harmony method or a PCR-ribotyping technique for methicillin sensitive (MSSA) strains. These genotyped isolates consisted of three MRSA isolates; MRSA1 (epidemic MRSA-15), MRSA100 (epidemic MRSA-16), MRSA546 (epidemic MRSA-15) and three MSSA isolates; MSSA51, MSSA55 MSSA62. *S. epidermidis* RP62A (ATCC 35984), a known biofilm-forming strain, was used as a positive control in biofilm assay and *S. aureus* National Collection of Type Cultures (NCTC) 6571 (Oxford strain) was used as a control for antibiotic susceptibility testing assays.

All isolates were stored in Microbank® storage vials (Pro-Lan Diagnostics) at -70°C and sub-cultured on brain heart infusion (BHI) agar (Oxoid, Basingstoke, UK) prior to each assay. Stock solutions were prepared by overnight incubation in BHI broth before 1ml of each bacterial suspension solution was centrifuged to create a pellet. 100µL of each pellet was diluted in BHI to a solution with an optical density (OD) of 0.03 for planktonic work.

Biofilm Formation

Biofilm formation was performed by the method described by Shanks et al (126). Cultures of staphylococcal sub-species NCTC 1, 100, 546, 51, 55, 62 and RP62 were incubated overnight in BHI broth before 5ml of each bacterial suspension solution was centrifuged to create a bacterial pellet of each organism. 100µL of each pellet was diluted in BHI to a solution with OD of 0.07 at 590nm. 150µL of each bacterial suspension was then added to a row of 12 individual wells of a 96-well microtitre dish. The final row of the 96-well microtitre dish was filled with 150µL of uninoculated BHI broth as a control. A corresponding 96-peg lid plate was then placed over the 96-well dish with each peg 'dipped' within its corresponding solution-filled well. The peg lid and well dish was then sealed and incubated at 37.0°C on a 'rocking' incubator tray for 48 hours. On removal from the rocking incubator the 96-peg lid was then washed in phosphate buffered saline to remove adherent cells with only biofilm remaining adherent to each peg.

Preparation of Antibiotic and Anticoagulant Solutions

A range of stock vancomycin solutions: 10,000mg/L, 1000mg/L, 100mg/L and 10mg/L, were prepared using sterile distilled water as a diluent, in accordance with the British Society for Antimicrobial Chemotherapy guidelines (127).

Using these stock solutions, each column of a 96-well microtitre dish was instilled with 75 μ L vancomycin solution of increasing concentration. Each well in column 1 contained 75 μ L 0.015mg/L vancomycin solution with the concentration of vancomycin doubled in each successive column up to a concentration of 16mg/L in column 11. Each well in the column 12 of the microtitre plate was filled with 75 μ L sterile water to act as control. These vancomycin-filled 96-well microtitre plates were used as the basis on which the experiments on planktonic bacteria were conducted.

Stock heparin solutions: 1000iu/mL and 2000iu/mL, were prepared using heparin sodium 1000iu/mL solution.

Three separate incubation media were created. Media A contained 80ml Mueller-Hinton (MH) broth and 20ml heparin 5000iu/mL solution, creating a MH-heparin 1000iu/mL broth. Media B contained 60ml MH broth and 40ml heparin 5000iu/mL solution, creating a MH-heparin 2000iu/mL broth. Media C consisted of 100ml MH broth without heparin. When conducting the analysis of combined vancomycin-heparin solutions, 75 μ L of heparin-based media was added to each well of the vancomycin-primed 96-well microtitre plates described above.

Planktonic MIC evaluation

To demonstrate the MIC of vancomycin against planktonic microbes, stock solutions of MRSA 1, 100, 546 and MSSA 51, 55, 62 and Oxford 6571 were prepared in the method described above to an OD of 0.3 at 590nm in Mueller-Hinton (MH) broth. These solutions were diluted by a factor of 1 in 10 and 75µL of this solution was added to each well of the pre-prepared vancomycin plates, thus equating with approximately 5×10^5 Colony Forming Units (CFU)/ml, the recommended inoculum for MIC assays (127). The experiment was repeated on a separate, duplicate 96-well vancomycin-inoculated dish. The dishes were then sealed and incubated overnight. After a 16-hour incubation period the OD of the well plates was read to assess the degree of bacterial growth.

To demonstrate the MIC of vancomycin-heparin against planktonic microbes, stock solutions of MRSA 1, 100, 546 and MSSA 51, 55, 62 and Oxford 6571 were prepared to an OD of 0.03 at 590nm using the MH-heparin 1000iu/mL (Media A) stock broth solution. 75µL of this solution was added to each well of the pre-prepared vancomycin plates. The dishes were then sealed and incubated overnight. After a 16-hour incubation period the OD of the well plates was read to assess the degree of bacterial growth. The experiment was then repeated using MH-heparin 2000iu/mL (Media B) stock broth solution. Both experiments were carried out in duplicate.

To demonstrate the effect of heparin on planktonic microbial growth, stock solutions of MRSA 1, 100, 546 and MSSA 51, 55, 62 and Oxford 6571 were then prepared to an optical density of 0.3 at 590nm in Mueller-Hinton (MH) broth. These solutions were diluted by a factor of 1 in 10 with 75µL of each solution then added to columns of eight duplicate wells

of a 96-well microtitre dish prepared with 75 μ L control MH broth alone, 75 μ L heparin 1000iu/mL solution (Media A) or 75 μ L heparin 2000iu/mL solution (Media B). A duplicate set of 96-well microtitre dishes were prepared, with both sets incubated overnight. After a 16-hour incubation period the optical density of the well plates was read to assess the degree of bacterial growth.

Biofilm MIC evaluation

Biofilm growth was conducted by the method described above using bacterial suspension of staphylococcal sub-species NCTC 1, 100, 546, 51, 55, 62, Oxford 6571 and the control strain RP62.

Each column of a separate 96-well microtitre dish was instilled with 75 μ L vancomycin solution in the method described above with each well in column 1 containing 75 μ L 0.25mg/L vancomycin solution and the concentration of vancomycin doubling in each successive column up to a concentration of 256mg/L in column 11. Each well in the column 12 of the microtitre plate was filled with 75 μ L sterile water to act as control. Each well was then inoculated with 75 μ L Media A containing the MH-heparin 1000iu/mL broth. The biofilm-containing peg plate was then dipped into the well plates and incubated on a rocking incubator tray for 16 hours.

To assess biofilm production, the prepared biofilm-impregnated 96-peg lids were dipped into 0.5% crystal violet solution with contact time of 2 minutes before gently washing with sterile water (figure 5.1). The 96-peg lid was then dipped into a 96-well plate, each well

containing 150 μ L 70% ethanol solution so as to leach the absorbed crystal violet from the cells. The absorbance of the resultant solution was measured at 570 nm using a microtitre plate reader (BMG LUMIstar* plate reader, BMG, Germany).

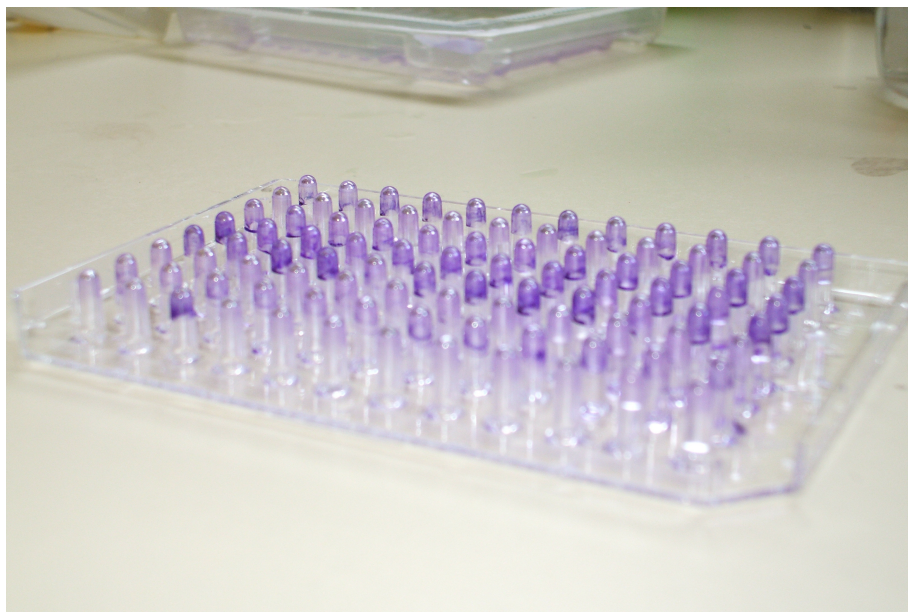


Figure 5.1 – An example of a biofilm-impregnated 96-peg lid having been dipped into 0.5% crystal violet solution for contact time of 2 minutes.

The experiment was then repeated using MH-heparin 2000iu/mL (Media B) stock broth solution and the heparin free MH broth solution (Media C). All experiments were carried out in duplicate.

Statistical Analysis

Statistical analysis was performed by SPSSTM version 14.0 (SPSS Inc, IL, USA). Parametric testing with student's t-test and non-parametric testing with the Wilcoxon-sign

rank test were used in the assessment of continuous variables where appropriate. All reported p-values were based on two-sided testing with a significance level set at $\alpha \leq 0.05$.

5.3 Results

In all the planktonic studies the MIC for the reference strain *S. aureus* NCTC 6571 Oxford strain was demonstrated at 1mg/L and was thus within one well dilution of the accepted MIC as determined by Andrews et al (127) and hence a valid control. In all the biofilm studies, the reference strain of *S. epidermidis* NCTC RP62 grew biofilm as demonstrated by a significantly greater OD than the MH control wells (see below).

Demonstrating the MIC of Vancomycin against planktonic microbes (pMIC)

With regard to the MRSA sub-species, the pMIC of vancomycin was found to be 2mg/L for NCTC 1 and 1mg/L for NCTC 100 and 546. With regard to the MSSA sub-species the pMIC of vancomycin was found to be 1mg/L for NCTC 51 and 55, and 2mg for NCTC 62.

Demonstrating the pMIC of Vancomycin-Heparin

When incubated in MH-heparin 500iu/mL solution, the pMIC of vancomycin was demonstrated at 0.5mg/L for MRSA NCTC 1, 100 and 546. With regard to the MSSA sub-species the pMIC of vancomycin was found to be 1mg/L for NCTC 51, 55 and 62.

Demonstrating the effect of Heparin on planktonic microbial growth

There was no significant change in OD following the addition of heparin to culture media for isolates of MSSA NCTC 55, 51 and MRSA NCTC 100.

There was a significant increase in OD of both heparin-containing culture media with *S. aureus* NCTC 6571 compared to the MH control (MH control OD=0.628 v MH-heparin 500iu/mL OD=0.695, $p=0.01$; MH-heparin 1000iu/mL OD=0.718 $p=0.001$).

MRSA NCTC 1 demonstrated a significant decrease in OD when incubated with MH-heparin 1000iu/mL culture media compared to MH-heparin 500iu/mL and MH control (MH-heparin 1000iu/mL OD=0.348 v MH-heparin 500iu/mL OD=0.419, $p<0.001$; MH control OD=0.408, $p<0.001$).

MRSA NCTC 546 demonstrated a significant decrease in OD when incubated with MH-heparin 1000iu/mL culture media compared to MH-heparin 500iu/mL and MH control (MH-heparin 1000iu/mL OD=0.164 v MH-heparin 500iu/mL OD=0.244, $p<0.001$; MH control OD=0.235, $p<0.001$).

MSSA NCTC 62 demonstrated a significant decrease in OD when incubated with MH-heparin 1000iu/mL culture media compared to MH-heparin 500iu/mL and MH control (MH-heparin 1000iu/mL OD=0.467 v MH-heparin 500iu/mL OD=0.646, $p<0.001$; MH control OD=0.597, $p<0.001$).

The comparison of OD between culture media for each bacterial isolate is demonstrated and summarised below in table 5.1.

Media	MRSA 1	MRSA 100	MRSA 546	MSSA 51	MSSA 55	MSSA 62	Oxford 6571
MH	0.408	0.504	0.235	0.546	0.684	0.597	0.628
MH + Heparin 500iu/mL	0.419	0.495	0.244	0.587	0.603	0.646	0.695 (p=0.01)
MH + Heparin 1000iu/mL	0.348 (p<0.001)*	0.487	0.164 (p<0.001)*	0.58	0.688	0.467 (p<0.001)*	0.718 (p=0.001)

Table 5.1 – Mean optical density at 590nm of suspensions incubated in MH, MH-heparin 500iu/mL and MH-heparin 1000iu/mL broth. P-values quoted refer to statistical comparison with MH control. *Significant difference in comparison with MH-heparin 500iu/mL group with p<0.001.

Assessment of Biofilm Production

Mean OD for the BHI broth control was 0.210. When compared to the BHI control the mean OD for each of the MRSA isolates was 0.253 for NCTC 1 (p=0.09), 0.318 for NCTC 100 (p<0.001) and 0.346 for NCTC 546 (p<0.001). For each of the MSSA isolates, the mean OD was 0.668 for NCTC 51 (p<0.001), 0.389 for NCTC 55 (p<0.001) and 0.920 for NCTC 62 (p<0.001). The mean OD for *S. epidermidis* RP62 was 0.288 (p=0.01) when compared with the BHI control. Full results are reported in table 5.2.

Bacterial Strain	1	2	3	4	5	6	7	8	9	10	11	12	Mean OD	P-value
Control	0.172	0.195	0.23	0.215	0.259	0.212	0.226	0.216	0.203	0.203	0.204	0.188	0.210	-
MRSA 1	0.167	0.205	0.392	0.397	0.199	0.234	0.156	0.263	0.268	0.188	0.244	0.327	0.253	p=0.09
MRSA 100	0.22	0.281	0.283	0.401	0.312	0.332	0.307	0.291	0.425	0.271	0.264	0.431	0.318	p<0.001
MRSA 546	0.251	0.358	0.33	0.249	0.516	0.345	0.328	0.252	0.461	0.385	0.31	0.363	0.346	p<0.001
MSSA 51	0.302	0.397	0.647	0.465	1.005	1.195	0.74	0.339	0.62	0.931	0.69	0.689	0.668	p<0.001
MSSA 62	0.885	0.87	0.465	1.716	2.545	0.801	1.075	0.617	0.544	0.537	0.72	0.262	0.920	p<0.001
MSSA 55	0.3	0.305	0.505	0.283	0.305	0.487	0.354	0.584	0.434	0.382	0.383	0.343	0.389	p<0.001
StaphRP62	0.208	0.22	0.248	0.445	0.267	0.448	0.198	0.206	0.24	0.38	0.245	0.354	0.288	p =0.01

Table 5.2 – Optical density at 590nm recorded in each well of a 96-well microtitre dish to assess biofilm formation of each bacterial strain in comparison with a control of BHI broth.

Demonstrating the MIC of Vancomycin-Heparin against microbes in biofilm (bMIC)

Biofilm production was assessed in MH broth. When assessing the bMIC of vancomycin against staphylococcal biofilms only MRSA NCTC 1 and 546 grew sufficient biofilm in the control wells to be suitable for assessment. The bMIC of vancomycin was determined at 4mg/L for MRSA NCTC 1 and 8mg/L for MRSA NCTC 546.

Biofilm production was then assessed in MH-heparin 500iu/mL broth. All staphylococcal strains produced biofilm in the control wells. The bMIC of vancomycin decreased to <0.25mg/L for MRSA NCTC 1 and decreased to 2mg/L for MRSA NCTC 546. The bMIC of vancomycin was determined at less than 0.25mg/L for MRSA NCTC 100 and MSSA NCTC 51, 55 and 62.

Biofilm production was then assessed in MH-heparin 1000iu/mL broth. All staphylococcal strains produced biofilm in the control wells. The bMIC of vancomycin remained <0.25mg/L for MRSA NCTC 1 and at 4mg/L for MRSA NCTC 546 was within one dilutional well of the MIC determined in MH-heparin 500iu/mL. The MIC of vancomycin was determined at less than 0.25mg/L for MRSA NCTC 100 and MSSA NCTC 51, 55, 62.

Full results for both pMIC and bMIC are demonstrated below in table 5.3.

Bacterial Strain	pMIC Vanc alone (mg/L)	pMIC Vanc/Hep 500iu/mL (mg/L)	bMIC Vanc alone (mg/L)	bMIC Vanc/Hep 500iu/mL (mg/L)	bMIC Vanc/Hep 1000iu/mL (mg/L)
MRSA 1	2	0.5	4.0	<0.25	<0.25
MRSA 100	1	0.5	-	<0.25	<0.25
MRSA 546	1	0.5	8.0	2.0	4.0
MSSA 51	1	1	-	<0.25	<0.25
MSSA 55	1	1	-	<0.25	<0.25
MSSA 62	2	1	-	<0.25	<0.25

Table 5.3 – The minimum inhibitory concentration (MIC) of vancomycin to each staphylococcal sub-species in the presence of vancomycin alone or heparin in combination with vancomycin. ‘pMIC’ minimum inhibitory concentration against planktonic bacteria. ‘bMIC’ minimum inhibitory concentration against biofilm-embedded bacteria.

5.4 Discussion

Several important themes arise from these data when relating them to the study of antibiotic lock solutions.

Whilst published work in animal models suggests that heparin has minimal impact on antimicrobial activity against staphylococci (123). These in-vitro data demonstrated some planktonic staphylococci to have decreased suppression of growth whilst others demonstrate heightened suppression of growth when heparin was added to incubation solution.

Interestingly when introducing a fixed concentration of heparin to a range of vancomycin concentrations, heparin appeared to lower the minimum inhibitory concentration (MIC) of vancomycin in several instances. The accepted convention in experiments to establish the MIC of antimicrobial solutions, however, allows the MIC to be reproducibly within one well dilution. Consequently, whilst a drop in MIC occurred in four out of six staphylococci examined, only MRSA NCTC 1 developed a greater than 2-well, and thus significant, dilution drop in MIC.

When considering biofilm MIC experiments, the addition of heparin again appeared to significantly lower the MIC of vancomycin in the cases of MRSA NCTC 1 and 564. The effect of heparin on the MIC of vancomycin of the other staphylococcal sub-species was unable to be fully assessed in this short study. When heparin 500iu/mL was compared with

heparin 1000iu/mL, the MIC of vancomycin was unchanged. This would suggest that if heparin does potentiate the efficacy of vancomycin, the effect would appear not necessarily to be dose dependent under these conditions.

Consequently, it can be concluded that under these conditions, heparin may independently heighten or suppress bacterial growth when used on its own, depending upon the staphylococcus studied. Across a range of vancomycin concentrations, the addition of heparin appears to have the effect of lowering the MIC in certain instances. This suggests that heparin could modulate bacterial growth when combined with the antimicrobial vancomycin. This hypothesis could be proven by performing viability counts when repeating these experiments, demonstrating whether there is an increased killing effect.

The second significant finding of interest was the demonstration of the absolute MIC values for planktonic staphylococci exposed to vancomycin being lower than those determined for biofilm embedded staphylococci exposed to vancomycin. Biofilm produces a physical barrier between antimicrobials and bacteria and thus is more resistant to eradication than planktonic bacteria. To eradicate biofilm embedded bacteria, the antimicrobial must penetrate the glycoprotein calyx before binding with the bacteria. The introduction of heparin appears to enhance the action of vancomycin. This could arise from either increased delivery of vancomycin to biofilm embedded bacteria, possibly through heparin neutralising the electrical barriers to vancomycin penetration, or increased delivery of bacteria to vancomycin, possibly through the negatively charged heparin preventing further biofilm formation or increasing bacterial release from biofilm (128).

All these findings, however, must be tempered by the methodological limitations of the study. This was a small study of only seven laboratory-grown staphylococcal isolates, albeit known biofilm producers and thus potential pathogens for catheter-related bacteraemia. An evaluation of staphylococcal isolates taken from cases of proven staphylococcal catheter-related bacteraemia would have greater clinical validity. Another important point to be considered is the relatively small number of duplicate experiments to ensure accuracy of the results. Whilst the model of biofilm colonisation on the 96-peg plates appeared effective and reproducible, in some instances the biofilm yield was poorer than expected and thus could be optimised by refinement of the technique with duplication of the experiments in greater numbers.

The use of vancomycin as the antimicrobial under study may also be seen as a limitation. The choice of vancomycin was primarily driven due to its use as the antimicrobial of choice within the Glasgow Royal Infirmary Renal Unit when empirically treating catheter-related bacteraemia. Since this study was designed, the development of vancomycin-resistant enterococci has led to gentamicin becoming a more commonly used antimicrobial for constructing catheter lock solutions. Our use of vancomycin may, therefore, be seen as being of lesser clinical relevance than initially intended.

Nonetheless the clinical relevance of these findings remains of interest. When using heparin alone, there may be varied degrees of infection risk depending on the type of staphylococcal pathogens expressed with a haemodialysis population. When considering combined antimicrobial-anticoagulant solutions, in clinical practice the catheter lock solution may be 5mLs in volume and contain 500mg of vancomycin, making an especially

high local concentration of antimicrobial targeted against the biofilm within the lumen of the catheter – several higher than the MICs of the organisms expected to be pathogenic. We now know, however, that these solutions leak into the systemic circulation where the volume of distribution may be as high as 5 litres, and bathes the outer portions of the catheter where biofilm may congregate. There may therefore be a theoretical disadvantage of these solutions – excellent intraluminal suppression of biofilm but at the expense of poor suppression of planktonic bacterial grown in the blood stream and biofilm production on the outside of the catheter derived from increasingly resistant populations of organisms. These theoretical limitations merit scrutiny as part of randomised controlled trials using bacteraemia, resistance patterns, adverse events, catheter thrombosis and mortality as outcomes over both short and long-term periods of follow-up.

5.5 Conclusion

Currently, the evaluation of potential antibiotic lock solutions in-vitro is widely applied but its execution variable. There is great heterogeneity in the types of organism studied, whether they are studied in planktonic or biofilm models, the type and dose of antimicrobial and the type and dose of anticoagulant. It is widely accepted that biofilm is the underlying substrate to catheter-related bacteria. The results of this work suggest that there are differing magnitudes of response to vancomycin and heparin between planktonic and biofilm-embedded staphylococci. Consequently, in-vitro assessment of antibiotic-lock solutions should be based uniformly on biofilm models, be centred on their response to combinations of the antibiotic and anticoagulant at clinically relevant doses and be

conducted against organisms commonly implicated in the pathogenesis of catheter-related bacteraemia. It should be on this platform that clinical evaluation of successfully performing catheter-lock solutions should be carried forward into in-vivo work.

Chapter 6

**Contrast-Enhanced Magnetic Resonance Venography of
Central Veins for Assessment of Haemodialysis Vascular Access
– A Six-Year Case Series**

6.1 Background

Patients with established renal failure (ERF) may undergo a large number of vascular access procedures during the course of their time on renal replacement therapy (RRT). One of the most frequently complicated vascular access procedures is insertion of a central venous catheter (CVC) (54, 129-131). CVCs are inserted under many different circumstances. Up to 40% of ERF patients present late and require urgent commencement on haemodialysis which may only be secured with the placement of a CVC. Many patients established on RRT may rely on CVC insertion when vascular access, in the form of an arteriovenous fistula or graft, has failed, is yet to be established or is immature.

Occult central venous stenosis and/or thrombosis may complicate what was initially thought a straightforward catheter insertion in up to 65% of patients (132) and in many cases leads to distortion of the central venous architecture. Furthermore, ERF patients have a tendency towards a hypercoagulable state arising from intrinsic defects in both platelet function and plasma factor abnormalities (133) which may contribute to limiting the lifespan of a CVC. In patients who have suffered from complications of CVC insertion, subsequent vascular access provision with catheters, fistulae and grafts may become technically more demanding.

Clinicians have been using imaging techniques to assist in vascular access provision for many years. One such technique, contrast-enhanced magnetic resonance venography (CE-MRV) using gadolinium chelates, has been available for some years now with proven speed, accuracy and reproducibility in the detection and evaluation of central venous thrombosis and stenosis (54, 134, 135). Recently CE-MRV has come under close scrutiny

following reports indicating an association between the administration of gadolinium-based contrast agents and the development of the rare condition nephrogenic systemic fibrosis (NSF) (55, 77, 136-138). The exact nature of this association is studied and described in detail in Chapter 7 of this thesis.

Current recommendations limit the use of gadolinium contrast agents to patients with a creatinine clearance of $>30\text{mls/min/1.73m}^2$ which has effectively prevented the use of CE-MRV in the established renal failure (ERF) population. The value of CE-MRV in these patients is acknowledged and thus the use of non-linear gadolinium chelates, contrast removal strategies and non-contrast based MR imaging are now being explored as possible methods of delivering imaging of the vascular tree to this vulnerable patient group.

Whilst the risk of developing NSF is now fully appreciated, the benefits CE-MRV has made to vascular access provision have yet to be formally quantified. The aim of this report is to clarify the contribution that CE-MRV made to the management of ERF patients in Glasgow Royal Infirmary over a 6-year period ending when the association with NSF was reported. The indications, findings and contributions made in a historical case series of CE-MRVs are described and the relationship between a number of routinely recorded parameters taken at the time of imaging and findings reported on CE-MRV is investigated.

6.2 Methods

Patients

A retrospective analysis of all patients who received haemodialysis for ERF and underwent gadolinium CE-MRV between 1st January 2000 and 1st January 2006 was performed. This cohort was derived by searching our renal unit electronic patient record for details of all patients who had received haemodialysis for ERF during this time period and had a record of having undergone magnetic resonance imaging at any time in the past. To ensure no eligible patients were overlooked, an additional search of the hospital Radiology Information System (RIS – CRIS3, Healthcare Software Systems, Mansfield Woodhouse, Nottinghamshire, UK) was conducted for all patients who had undergone CE-MRV and this list cross-referenced with the renal unit records of ERF patients.

Clinical data from the date at which CE-MRV was undertaken was retrieved from the renal unit electronic patient record and recorded for each patient. This included records of age, blood pressure, presence of diabetes mellitus, underlying cause of renal failure, duration of renal replacement therapy, haemodialysis blood flow, urea-reduction ratio and the number and type of vascular access procedures that had been undertaken prior to CE-MRV. From review of both the radiology records of the CE-MRV studies and the electronic patient records, the initial indications for requesting CE-MRV in each case were recorded as either; 1) investigation of clinically suspected venous stenosis, thrombosis and/or venous

occlusion, or 2) a search for suitable site for vascular access provision.

CE-MRV

All examinations were performed on a 1.5 T MRI system (Gyrosan ACS NT, Philips Medical Systems, Best, The Netherlands) with the patient supine using a 4-element phased array body coil for signal reception over the thorax and upper arms in anatomical position (a minority of patients underwent imaging of the abdomino-pelvic veins with coils over the abdomen and pelvis in a similar manner). The exact details of the CE-MRV protocol evolved over the course of the study period consequent upon hardware and software upgrades. CE-MRV was always performed using a breath-hold coronal multiphase gadolinium contrast-enhanced technique with first acquisition timed using bolus tracking to systemic arterial phase upon contrast arrival in the aortic arch for thoracic studies. At least two successive breath-hold acquisitions were subsequently performed for equilibrium / venous phases, the first as soon after the first arterial dynamic phase as repeat breath-holding would allow and the last within 3 minutes of contrast agent injection. In essence this is an indirect CE-MRV technique imaging the central veins in phases after vascular distribution.

Consultant vascular radiologists with a specific interest in MR angiography interpreted all CE-MRV studies on a dedicated MRI workstation (EasyVision & ViewForum, Philips Medical Systems, Best, The Netherlands).

Outcomes

Radiological outcomes were derived from the radiologist's report of the images and categorised as to whether there was evidence of venous thrombosis alone, venous stenosis alone, venous thrombosis and stenosis in combination or whether the scan did not demonstrate any such abnormality.

Clinical outcomes were sought from the renal unit electronic patient record which was interrogated to determine what subsequent vascular access management had been instituted in the period following CE-MRV as derived from clinic letters, discharge summaries and records of vascular access intervention. Findings were summarised and recorded on the basis of whether following CE-MRV there was an *immediate* change in vascular access management (defined as within 2 weeks of CE-MRV), a *future* change in vascular access management (greater than 2 weeks and less than 6 months post CE-MRV) or whether *no change in management* occurred within 6 months of CE-MRV.

In light of the subsequently published association between gadolinium contrast agents and NSF in this group of patients (55, 77, 136-138) any instances of NSF were deliberately sought in this cohort by review of all case records and related pathology reports.

Interpretation of Outcomes

Tests of association between the outcomes of venous stenosis and venous thrombosis and the clinical and laboratory variables recorded at the time of CE-MRV were conducted by parametric and non-parametric testing as appropriate. Students t-testing and Mann-Whitney U testing were used in the analysis of continuous variables and Chi-square testing of categorical variables. For all statistical analyses the reported p-values were based on two-sided testing with a significance level set at $\alpha \leq 0.05$.

6.3 Results

Patient Data

During the period 2000-2006 the renal unit and associated satellite haemodialysis units referred 62 patients in whom a total of 78 CE-MRVs were performed. All CE-MRVs were requested to assess the central veins and aid in management of haemodialysis vascular access. A total of 29/62 (46.8%) patients were male and 33/62 (53.2%) female with mean age 58.9yrs (16.14 SD). 18/62 (29.0%) patients were known diabetics. Median length of time on renal replacement therapy was 804 days (range 26, 5982) (table 6.1).

All patients had chronic renal failure requiring RRT with 12/62 (19.4%) having a primary glomerulonephritis, 17/62 (27.4%) an interstitial nephritis, 8/62 (12.9%) renal failure as

part of a multi-system disease, 10/62 (16.1%) with diabetic nephropathy and 15/62 (24.2%) with a non-specified or unclassifiable cause for their renal failure.

Variable	Mean (SD)
Age (years)	58.9 (16.1)
Male / Female	29/62 (46.8%) / 33/62 (53.2%)
Diabetes	18/62 (29%)
Duration of RRT (days)	804 (26, 5982)*
Haemodialysis blood flow (ml/min)	255.8 (59.9)
Urea reduction ratio (%)	67.5 (7.3)
MABP (mmHg)	96.6 (14)
AVF / Graft creations	2 (0,8)*
CVC insertions	5 (0, 22)*

Table 6.1 – Baseline characteristics of the cohort immediately prior to undergoing CE-MRV. MABP = mean arterial blood pressure, AVF = arteriovenous fistula, CVC = central venous catheter. *Median (min, max).

Of the patients studied, arteriovenous fistula or graft surgery had been undertaken on a median of 2 (range 0 to 8) occasions per patient prior to CE-MRV (figure 6.1). Successful central venous catheterisation had been undertaken on a median of 5 (range 0 to 22) occasions prior to CE-MRV (figure 6.2).

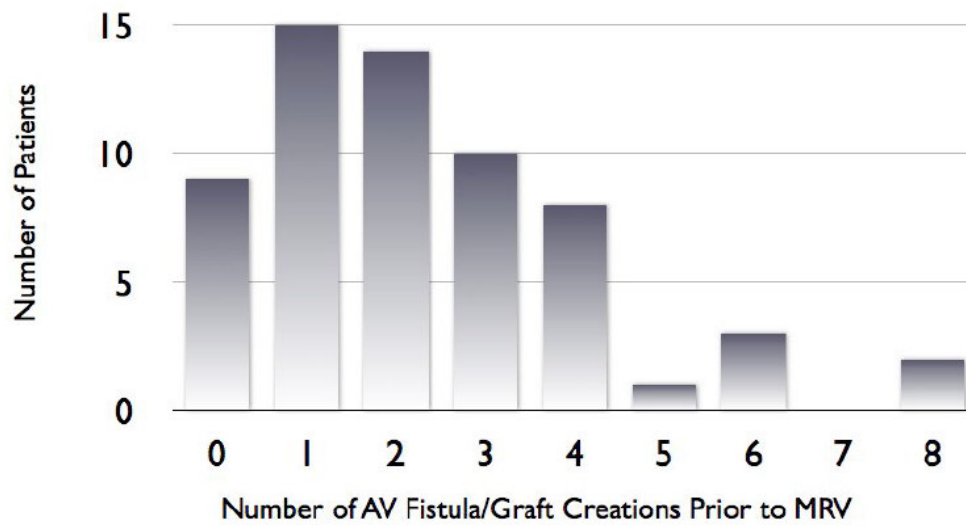


Figure 6.1 – Frequency plot of arteriovenous (AV) fistula and arteriovenous graft creation procedures undertaken prior to CE- MRV.

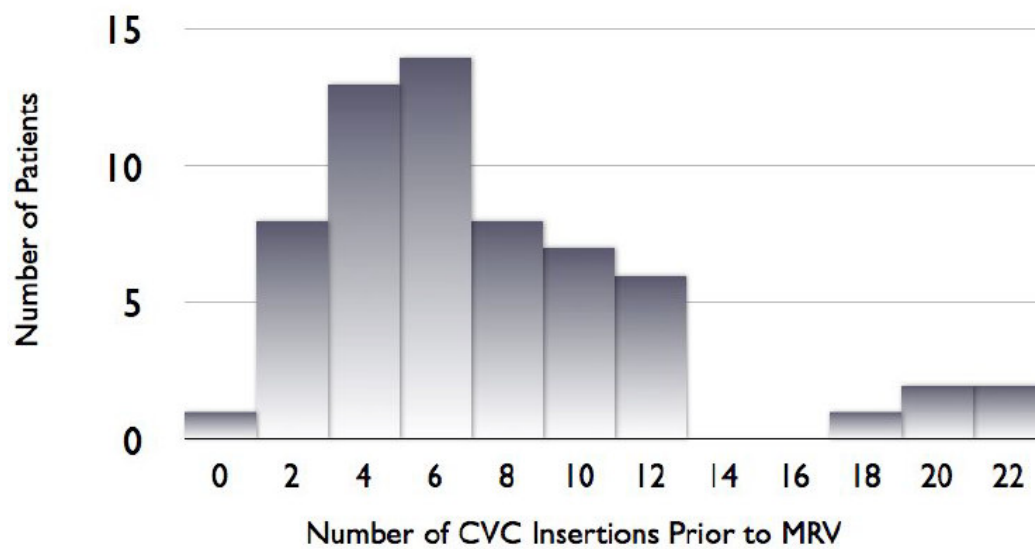


Figure 6.2 - Frequency plot of number of central venous catheters inserted per patient prior to CE-MRV.

Indications for CE-MRV

Of the 78 CE-MRVs undertaken, the majority (72/78, 92.3%) were for assessment of the central veins of the neck and thorax with only a minority (6/78, 7.7%) studying the femoral and iliac veins plus inferior vena cava. In a total of 46/78 (59.0%) investigations CE-MRV was requested in patients in whom there was clinical suspicion of veno-occlusive disease secondary to previously established vascular access. 32/78 (41.0%) CE-MRVs were performed in order to establish suitable sites for future vascular access provision.

Radiological Outcomes

Of the 78 CE-MRVs undertaken 32/78 (41.0%) demonstrated venous stenosis alone, 6/78 (7.7%) demonstrated venous thrombosis alone, 20/78 (25.6%) demonstrated both localised venous stenosis and venous thrombosis within the large veins and 20/78 (25.6%) demonstrated no abnormalities of the central venous system.

In patients where CE-MRV confirmed venous stenosis, univariate testing demonstrated a significant association with increased incidence of surgery for arteriovenous fistulae or synthetic arteriovenous grafts (stenosis=2.0 v no stenosis=1.0, $p=0.040$) and central venous catheterisation (stenosis=6.0 v no stenosis=4.5, $p=0.034$). There was no association between venous stenosis and patient age, gender, urea-reduction ratio, haemodialysis flow, mean arterial blood pressure, duration on renal replacement therapy or the presence or absence of diabetes mellitus.

In patients where CE-MRV confirmed venous thrombosis, univariate testing demonstrated significantly lower haemodialysis flow rates (thrombosis 217.5mls/min v no thrombosis 275mls/min, $p=0.002$) and a higher mean monthly arterial blood pressure (thrombosis 101.5mmHg v no thrombosis 94.2mmHg, $p=0.046$). There was no association demonstrated between venous thrombosis on CE-MRV and the number of previous arteriovenous fistulae or grafts, the number of previous central venous catheters, patient age, gender, urea reduction ratio, duration on renal replacement therapy or the presence or absence of diabetes mellitus.

Clinical Outcomes

In 36/78 (46.2%) of cases changes to vascular access management occurred within a two week period of CE-MRV. In nearly 30 cases this was achieved through the insertion of a tunnelled central venous haemodialysis catheter, in 3 cases through percutaneous angioplasty/stenting of the central veins, in 2 cases patients were anticoagulated and in 1 patient previously scheduled endovascular intervention was prevented from being unnecessarily attempted.

In 27/78 (34.6%) of cases changes to vascular access management occurred within the period from two weeks to six months following CE-MRV.

In 15/78 (19.2%) cases there was no documented evidence from the patient records that CE-MRV had directly influenced subsequent patient management.

Patients who had abnormalities on MRV were more likely to undergo vascular access intervention in the 6 months following imaging than those with a normal CE-MRV (Abnormal MRV 50/58 (86.2%) v Normal MRV 12/20 (60%), $p=0.031$).

From the whole cohort of 62 patients 4 were identified as having developed suspected NSF (51, 70, 128-130), each had received 30 ml gadodiamide for their CE-MRV studies or other MRI investigations (in one patient on 3 occasions in total) prior to the onset of symptoms attributed to NSF. No patients in our series receiving gadobenate dimeglumine (Multihance) alone are suspected of having developed this condition to date. These cases are analysed in detail as part of a case-control study described in Chapter 7 (55).

6.4 Discussion

Arteriovenous fistulae are widely recognised as the gold standard in haemodialysis vascular access due to their lower rate of infective and thrombotic complications when compared to central venous haemodialysis catheters (139). However, in settings where interim haemodialysis is required the advantage conferred by central venous catheterisation as a means of gaining quick, durable and reliable access to the vascular tree often outweighs these relative disadvantages. Consequently central venous catheterisation remains a cornerstone in the maintenance of patients on haemodialysis. Central venous catheterisation, however, may give rise to structural complications which may impact on the provision of future vascular access. The demographics of this cohort describe such a

group. In general, the majority of patients studied had been on renal replacement therapy for many months or years, had been exposed to multiple vascular access procedures and in whom the maintenance of vascular access had become actively problematic. In the vast majority of cases CE-MRV gave a definitive assessment of venous patency that had proven difficult to quantify by clinical or other radiological means.

The methodology used was observational, retrospective and in a select group of problematic patients and thus has to be taken carefully in this context. No control group was studied and thus these findings are purely observational and without any direct comparator. Patient selection, the pattern of vascular access usage, the threshold for imaging and the availability of CE-MRV are features that will vary across different haemodialysis patient populations. As such, MRV findings reported in this group may not be directly transferable to other haemodialysis populations and have to be observed within these limitations. Despite these issues several important themes have been demonstrated.

A total of 74% of CE-MRVs demonstrated abnormalities in the form of venous thrombosis, venous stenosis or both. Patients with an abnormal CE-MRV were significantly more likely to have successful vascular access establishment in the 6 months following scanning than those with a normal CE-MRV. This included instances where CE-MRV demonstrated positive findings that led to percutaneous venous angioplasty and stent insertion in three cases. In another instance a patient was spared previously planned percutaneous venous angioplasty and stent insertion after CE-MRV failed to demonstrate a suitable central venous anatomical abnormality. Negative findings on CE-MRV for the central veins also proved useful with several patients who would have been considered for anticoagulation

being spared unnecessary treatment. These findings suggest that the anatomical data generated by CE-MRV was an important contributor in the management of vascular access in this patient group.

This case series also studied trends of association between baseline clinical variables and the vascular access complications found on CE-MRV. The findings were consistent with the published literature to date, albeit in patient populations without ERF. The association between low haemodialysis blood flow and central venous thrombosis is to be expected in an analysis such as this with low haemodialysis blood flow likely to be both a cause and effect of localised central venous thrombosis. What is more difficult to explain is the positive association between an increased mean arterial pressure and venous thrombosis. A potential explanation is that elevated mean arterial pressure is a marker of underlying cardiovascular disease and is hence associated with both vascular endothelial dysfunction and an underlying thrombogenic state. This is an area that is not addressed by this particular study design but would merit future investigation.

A further interesting finding is a positive association between the number of previous arteriovenous fistulae or synthetic graft procedures and the development of central venous stenosis. These methods of vascular access often deliver high velocities of turbulent blood flow back into the venous tree that could theoretically give rise to local vascular endothelial dysfunction and create a predisposition to an increased thrombogenic state. A prospective examination of the relationship between arteriovenous fistulae or synthetic grafts, markers of vascular endothelial function and the development of mechanical complications such as venous thrombosis and stenosis would be required to investigate this. Such work would help build a definition of the ‘at-risk’ patient and therefore identify those most likely to

derive benefit from vascular access planning and targeted intervention.

Several reports have compared the utility of CE-MRV with venography and ultrasound in patients who have required vascular access to administer fluid, chemotherapeutic drugs or nutrition or who have been clinically suspected of having central vein thrombosis (140-145). These reports indicated CE-MRV to be superior in demonstrating differing degrees of venous stenosis, thrombosis and anatomical variation when compared to ultrasound (140-142). In comparison with traditional venography, CE-MRV was found to be equivalent in assessing venous patency and better at demonstrating venous anatomy and blood flow whilst being less invasive, avoiding radiation and avoiding exposure to potentially nephrotoxic iodinated contrast (141-145). The finding of venous stenosis in 66.6% of the study population is approximately equitable to the prevalence of venous stenosis seen in similar patient populations who have used venography (132). Differing prevalence of stenosis and thrombosis in such studies may be accounted for by patient selection, different patterns of vascular access usage, differing thresholds for imaging and variability in access to imaging.

CE-MRV was however found to be associated with significant health risk with four patients within the cohort being subsequently found to have developed NSF. These patients underwent gadolinium contrast-enhanced studies prior to the issuing of the FDA and ACR guidelines on the use of gadolinium-based contrast agents in patients with renal dysfunction (146, 147). The association between the administration of different gadolinium contrast agents in patients with ERF and the development of NSF was clearly of concern and was investigated further, the details of which are described in the next chapter.

Clearly, however, CE-MRV would appear to have been of value in the majority of patients in this cohort. Alternatives to traditional gadolinium CE-MRV where the risk of NSF is minimised and the quality of anatomical data generated preserved, should be explored. This may be achieved by focussing on the use of non-linear gadolinium chelates, contrast removal strategies and non-contrast based MRI. These methods are now being explored as possible means of imaging the vascular tree to this vulnerable patient group.

6.5 Conclusion

These data have demonstrated that CE-MRV has been an effective imaging modality in the assessment, planning and maintenance of haemodialysis vascular access with central venous catheters. Use of CE-MRV has been shown to be associated with significant changes to vascular access management and clinical decision-making whilst offering what appeared until recently to be a more acceptable side-effect profile than the previous gold-standard technique of contrast venography in patients with renal failure. These advantages, however, must now be considered against the risk association with the use of specific gadolinium-based contrast agents in ERF. Only once the NSF issue is resolved can CE-MRV have an effective role in patients with problematic vascular access.

Chapter 7

Gadolinium-Enhanced Magnetic Resonance Imaging and Nephrogenic Systemic Fibrosis – A Retrospective Study of a Renal Replacement Therapy Cohort

7.1 Background

Nephrogenic systemic fibrosis (NSF) (previously labelled nephrogenic fibrosing dermopathy) was first described in 2000. The earliest published cases described a scleromyxoedema-like condition affecting patients with advanced kidney disease. Typically, the condition presents cutaneously with thickening, oedema, induration or discolouration of the skin of the limbs and trunk, although most commonly with facial sparing. Progressive disease may result in joint contractures and subsequent loss of limb function. Fibrosis of other organ systems may occur with resultant end-organ dysfunction (148-150). Mortality has been noted to be high although anecdotal improvement in both morbidity and mortality has been reported following recovery of renal function, either spontaneously or after renal transplantation, or with immunomodulatory treatments including extracorporeal photophoresis and phototherapy (151-154).

The diagnosis of NSF was made initially on clinical grounds alone although histological features which may support the diagnosis are now widely accepted. Skin lesions demonstrate dermal thickening, which stains positive for mucin, and with collagen bundles which traverse down into the superficial fascia (155). Fibrous bundles contain CD34/procollagen expressing fibroblast-like cells and CD68/FactorVIIIa expressing dendritic-like cells. The combination of these typical pathological and laboratory features distinguishes NSF from similar conditions including systemic sclerosis, scleromyxoedema and eosinophilic vasculitis. Indeed, to fully confirm a diagnosis of NSF, the absence of scl70 and anti-centromere antibodies as well as a normal serum electrophoresis (136, 156) should be obtained.

To date, 304 patients have been reported to the NSF registry (155, 157). Demographically, these patients have been found to be similar to the general established renal failure (ERF) population although mean age is lower (136, 158). Vascular disease, thrombotic events, chronic liver disease and the presence of anti-phospholipid antibodies have been associated with NSF, although not directly implicated in its pathogenesis (156, 159-163).

Since 1998 the increasing use of gadolinium-enhanced magnetic resonance imaging (MRI) in ERF to avoid iodinated contrast exposure has paralleled the description of patients with NSF. Several case series have since explored the association between NSF and exposure to gadolinium containing contrast agents, reporting a relatively high frequency of gadolinium-enhanced MRI in patients prior to developing NSF (77, 164-168).

This retrospective study was designed to compare the frequency of administration and cumulative dose of gadolinium contrast agent in a cohort of dialysis dependent patients who did and did not develop NSF.

7.2 Methods

Patients

A retrospective analysis of all patients who received renal replacement therapy (RRT) for established chronic renal failure (Stage V Chronic Kidney Disease, eGFR<15mls/min) within the renal units of two city teaching hospitals and associated satellite units in the West of Scotland, between 1st January 2000 and 1st July 2006 was conducted. Patients were analysed as to whether they had a diagnosis of NSF and with regards their previous exposure to gadolinium contrast-enhanced MRI. The local ethics committee granted a waiver of review for the study and also waived the need for informed consent.

The patient cohort was obtained through a search of the unitary electronic patient record (EPR), a computerised database containing details of all patients who have ever been referred to either of the two units. An EPR search was conducted to determine all patients within the two hospitals who had undergone RRT for stage V CKD between 1st January 2000 and 1st July 2006. Patients who had functioning renal grafts throughout the study period and thus did not receive dialysis and all patients who received RRT for acute renal failure for less than 90 days were excluded. Age, gender, underlying renal diagnosis, time on RRT, mode of RRT and mortality were recorded from the EPR for each patient in the cohort.

Database Interrogation

Diagnosis entries on the EPR for all patients on RRT with stage V CKD were then searched for the terms “nephrogenic”, “systemic”, “fibrosis”, “fibrosing”, “dermopathy”, “scleroderma”, “scleromyxoedema” and “sclerosis”. All patients with a positive match for any of these search terms were reviewed thoroughly with respect to the clinical history and histopathological records to determine those patients with a diagnosis of NSF. Where the original biopsy specimens were available they underwent further review by a pathologist to ensure the validity of the original diagnosis. Age at starting RRT, age at diagnosis, sex, cause of renal failure, dialysis modality, time on RRT, the presence of a confirmatory skin biopsy, serum bicarbonate (mmol/l) at time of MRI, weight (kg) at time of MRI, subsequent treatment, whether they recovered renal function, clinical outcome, duration of follow-up and the time period between imaging and a subsequent diagnosis of NSF were recorded for each NSF patient.

The EPR was then interrogated with regard to the cohort’s previous exposure to MRI. The type of MRI scanner used, number and type of gadolinium-enhanced MRI studies, cumulative gadolinium dosage (mls), cumulative gadolinium dosage adjusted for weight (mmol/kg), average dose per scan (mmol/kg/scan) and type of gadolinium chelate was recorded for each patient. Where complete data was not provided in the radiology reports held on the EPR, hand searching of the radiology departmental records was conducted. Gadolinium exposure was calculated over the six and a half-year period for all non-NSF patients and for the period 1st January 2000 until the first presentation of disease for all patients in the NSF group. Weight adjusted dose was calculated by using the dry weight at

the time of imaging in the case of haemodialysis patients or recorded weight within six weeks of imaging. The number of MRI scans undertaken prior to and following the diagnosis of NSF was recorded for each NSF patient. The cumulative frequency of gadolinium-enhanced MRI and NSF cases was recorded for the study period.

Statistical Analysis

Statistical analysis was performed by SPSSTM version 14.0 (SPSS Inc, IL, USA). Shapiro-Wilk testing for normality was performed on continuous variables with rejection of the null hypothesis of normality at a significance level of $\alpha \leq 0.05$. Testing for equality or homogeneity of variance was conducted using Levene's tests.

Difference in mean age between NSF and non-NSF patients was assessed by Students' t-test. Prevalence of gadolinium-enhanced MRI exposure between NSF and non-NSF groups was assessed by Chi-square testing. Differences between median number of gadolinium-enhanced MRI, median cumulative dose of gadolinium contrast (mls and mmol/kg) and median dose of gadolinium contrast (mls/kg/scan) in NSF and non-NSF groups were assessed by Wilcoxon rank sum testing. Differences in gender and mortality were assessed by Chi-square testing. All reported p-values were based on two-sided testing with a significance level set at $\alpha \leq 0.05$.

7.3 Results

Patients

1826 stage V chronic kidney disease patients who had received RRT for ERF in North Glasgow Renal Units during the study period were identified. The mean age at starting RRT was 58.8 years and 1053/1826 (57.7%) patients were male. In total, 253/1826 (13.9%) commenced RRT due to ERF as a result of a primary glomerulopathy, 374/1826 (20.5%) with an interstitial nephropathy, 336/1826 (18.4%) with a multi-system disease, 281/1826 (15.4%) with diabetic nephropathy and 582/1826 (31.9%) with an unknown or unclassifiable cause of their renal failure.

MRI characteristics

Over the 6 and a half-year period, 421/1826 (23.1%) patients underwent a total of 542 gadolinium-enhanced MRI. Examinations performed using contrast-enhanced MRI included angiography (430/542, 79.3%), cardiac studies (90/542, 16.6%) and central nervous system imaging (22/542, 4.1%). A total of 379/542 (69.9%) contrast enhanced MRI examinations were performed on 1.5T superconducting systems whilst 163/542 (30.1%) were performed on 1.0T superconducting systems. Most examinations were assigned 30-minute appointments except for cardiac studies, which were usually scheduled 1.5 hours. However, the exact time duration for static RF field and pulse sequence varying fields would have been variable and was not recorded. 480/542 (88.6%) MRI scans were

performed with gadodiamide (Omniscan, GE Healthcare, Chalfont St Giles, UK) with a median dose volume of 30ml, reflecting the predominant use of this agent in the West of Scotland since 1998. 62/542 (11.4%) scans were performed using other gadolinium-containing contrast agents as follows: 41/542 (7.6%) MRI used gadobenate dimeglumine (Multihance, Bracco S.p.A., Milan, Italy) as the contrast agent with median dose volume of 15ml. 13/542 used gadopentetate dimeglumine with a median dose of 15mls (Magnevist, Berlex, Canada), 6/542 gadobutrol with a median dose of 15mls (Gadovist, Schering, West Sussex, UK) and 2/542 gadofosvest trisodium with a median dose of 10mls (Vasovist, Schering, West Sussex, UK). The annual incidence of gadolinium-enhanced MRI among the cohort steadily increased until 2004 with fewer scans undertaken in 2005 and a subsequent reduction in contrast-enhanced MRI in patients on RRT in response to the FDA warning issued in June 2006. The incidence of NSF parallels the rise in MRI imaging in the RRT cohort and is demonstrated in figure 7.1.

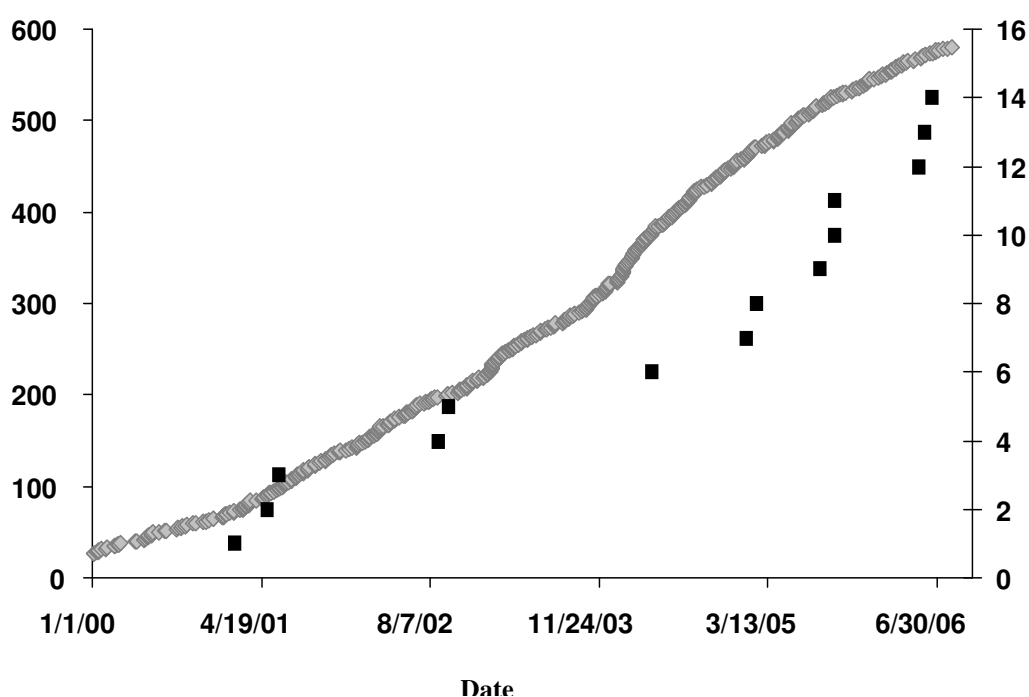


Figure 7.1 - Frequency of MRI and NSF incidence from January 2000 to July 2006 (x-axis), number of gadolinium-enhanced MRIs (line) and incidence of NSF (square dots). The increasing use of MRI parallels the identification/development of patients with NSF.

NSF versus non-NSF cases

The clinical and laboratory characteristics of the patients with NSF are demonstrated below in table 7.1

Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sex	M	F	M	F	M	F	F	M	F	F	M	F	F	F
Primary diagnosis	U	P	DM	GN	DM	GN	U	AP	DM	P	DM	P	GN	GN
Skin biopsy	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y
Age (years)	63.2	61.0	43.5	54.3	67.1	49.0	63.7	43.7	37.7	20.5	60.4	58.5	59.8	73.2
Age starting RRT (years)	61.6	56.6	42.4	22.2	67.0	49.0	56.7	38.9	35.1	17.0	59.9	51.7	59.1	66.2
Time on RRT (years)	1.6	4.4	1.1	32.1	0.1	0.0	7.0	4.8	2.6	3.5	0.5	6.8	0.7	7.0
RRT mode	PD	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD	PD	HD
HCO ₃ (mmol/l)	22	24	26	25	18	28	18	36	17	25	23	26	29	21
Photophoresis		Y			Y									
Renal function	-	-	-	-	-	Sp	-	-	-	-	-	Tx	-	-
Follow up (days)	691	2006	72	105	80	1863	178	252	30	460	257	799	533	50
Outcome	S	I	D	D	S	I	D	D	na	D	D	S	S	na

Table 7.1 - Baseline characteristics of NSF patients. M, male; F, female; U, unknown; P, pyelonephritis; DM, diabetes mellitus; GN, glomerulonephritis; AP, adult polycystic kidney disease; Y, yes; N, no; Time on RRT, at diagnosis with NSF; RRT mode, at the time of NSF diagnosis; PD, peritoneal dialysis; HD, haemodialysis; HCO₃, serum bicarbonate level within 30 days of MRI; -, no change; Sp, spontaneous improvement; Tx, renal transplant; Outcome, of NSF; S, stable; I, improved; D, dead; na, not applicable/follow up time too short for comment.

From the full cohort, a total of 14/1826 (0.77%) patients had an established diagnosis of NSF as evident by clinical and pathological findings noted within the clinical records and on further review of the biopsy material where available. Despite skin biopsy not being

performed in two patients the clinical picture, based on examination and laboratory tests, was considered consistent with the diagnosis of NSF by both a dermatologist and nephrologist. With the caveat that the NSF group were relatively few in number in comparison to the non-NSF group, the mean age at starting RRT was significantly lower for NSF patients at 48.3yrs (p=0.022) and the median time on RRT at diagnosis was 3 years. A comparison of the characteristics of patients with NSF and without-NSF is detailed in table 7.2 below.

Characteristic	NON-NSF	NSF	Significance Level
Number of patients	n=1812	n=14	-
Age	58.8yrs (16.9)*	48.3yrs (15.1)*	p=0.022
Prevalence of Gadolinium MRI	408/1812 (22.5%)	13/14 (92.9%)	p<0.001
EDTA Diagnosis			
Primary Glomerulonephritis	n=249/1812 (13.7%)	n=4/14 (28.6%)	-
Interstitial Nephropathy	n=370/1812 (20.4%)	n=4/14 (28.6%)	-
Multisystem Disease	n=336/1812 (18.5%)	n=0/14	-
Diabetic Nephropathy	n=277/1812 (15.3%)	n=4/14 (28.6%)	-
Unknown/Unclassifiable	n=580/1812 (32.0%)	n=2/14 (14.3%)	-
Proportion Male:Female	1.37	0.55	p=0.095
Mortality at end of study period	707/1812 (39.0%)	6/14 (42.9 %)	p=0.77

Table 7.2 - Clinical characteristics of the complete RRT cohort comparing those with a diagnosis of NSF to those without. All variables expressed as value (%). *Variable expressed as mean (SD).

The prevalence of and relative dosing of gadolinium-enhanced MRI within the cohort and amongst NSF patients is described in tables 7.3 and 7.4 respectively.

Characteristic	NON-NSF	NSF	Significance Level
Prevalence of Gadolinium-MR	n=408	n=13	P<0.001
Gadodiamide Dose (ml)	30	45	P<0.001
Gadodiamide Dose (mmol/kg)	0.23	0.39	P=0.008
Gadodiamide Dose (mmol/kg/scan)	0.21	0.20	P=0.83
Scan Number	1 (1, 6)	2 (1, 3)	P=0.009
MRA/V	323/455 (71.0%)	16/25 (64.0%)	P=0.46

Table 7.3 - Pattern of MRI and gadodiamide dosage for the complete RRT cohort. Gadodiamide doses and scan number are given as medians. MRA/V, proportion of MRI performed for angiography.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14
MRI Imaging	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Total Dose (ml)	90	90	40	na	30	30	90	45	45	60	45	40	58	75
Total Dose (mmol/kg)	0.60	0.47	0.20	na	0.16	0.30	0.61	0.41	0.39	0.90	0.27	0.21	0.39	0.65
Dose (mmol/kg/scan)	0.20	0.16	0.20	na	0.16	0.30	0.20	0.41	0.39	0.45	0.27	0.21	0.19	0.13
MRI-NSF time (days)	76	83	477	na	2395	35	2	4	16	52	64	289	99	441
MRI pre NSF	3	3	1	0	1	1	3	1	1	2	1	1	2	5
MRI post NSF	1	2	0	0	1	1	1	1	0	3	0	0	1	0

Table 7.4 - Pattern of MRI and gadodiamide dosage within NSF patients. Y, yes; N, no. Total median dose of gadodiamide administered prior to onset of NSF is given in ml and mmol/kg. Median gadodiamide dose per scan is given in mmol/kg/scan. MRI-NSF time is the time interval between gadolinium-enhanced MRI and NSF onset; MRI pre NSF is the total number of MRI scans prior to NSF diagnosis; MRI post NSF is the number of MRI scans performed after a diagnosis of NSF.

13/14 patients with NSF had undergone gadolinium-enhanced MRI, all having been dosed with gadodiamide (tables 7.3 and 7.4). None of the NSF patients had been exposed to any of the other gadolinium-based contrast agents. One patient (patient 4) in our study developed biopsy proven NSF without prior exposure to gadolinium. This patient had been on renal replacement therapy for 32 years, had previous thrombotic vascular disease (diagnosed by conventional angiography) and was known to have chronic hepatitis C infection. An extensive review of her past medical history was undertaken to ensure no gadolinium-enhanced MRI had been undertaken. This patient died within 3 months of the diagnosis of NSF and thus direct questioning of the patient could not be performed.

Analysis of the full cohort demonstrated a strongly significant association between undergoing gadolinium-enhanced MRI and the subsequent development of NSF (table 7.3, non-NSF 408/1812, 22.5% v NSF 13/14, 92.9%, $p<0.001$). The relative risk of developing NSF following gadolinium-enhanced MRI was 43.38 ($\{13/421/\{1/1405\}\}$). 13/421 (3.1%) of the ERF population exposed to gadodiamide developed NSF with 14/1826 (0.77%) of the RRT population having developed NSF. When considering the 421 patients who had undergone gadolinium-enhanced MR, patients with a diagnosis of NSF were exposed to a significantly higher total dose of gadodiamide than their non-NSF counterparts (median 45ml v 30ml, $p<0.001$). This was supported by weight-adjusted data (median 0.39mmol/kg v 0.23mmol/kg, $p=0.008$). Weight data was incomplete for 19 patients, all within the non-NSF group, which were excluded. Patients underwent a median of 1 scan per patient in both groups, however the distribution of scanning events demonstrated a greater proportion of gadolinium scans within the NSF group ($p=0.009$). No difference was found in the average dose of gadodiamide per scan between the groups (median 0.20mmol/kg NSF v

0.21mmol/kg non-NSF, $p=0.83$) nor in the proportion of gadodiamide scans undertaken for angiography (16/25, 64% NSF v 323/455, 71% Non-NSF) (table 7.3).

The time between gadolinium exposure and presentation with NSF ranged from 2 to 2395 days (median time 76 days). Five patients developed NSF greater than 90 days after receiving gadodiamide. Of these, three had a time of onset greater than one year. Six patients within the NSF group had undergone previous gadolinium-enhanced MRI without apparent complication and eight went on to receive further exposure after presentation with NSF.

Outcome of NSF Patients

Within the full cohort no significant difference between NSF and non-NSF groups was found with regard to mortality ($p=0.77$). At the end of the study period 6/14 (42.9%) of patients with NSF had died however, the follow-up period for four of the remaining eight patients was less than three months. The median time to death following diagnosis of NSF was 215 days. In three patients the cause of death was recorded as sepsis-related occurring in hospital, two patients suffered an in-hospital cardiac arrest (one in the context of hyperkalaemia) and one patient died suddenly at home, all without subsequent post-mortem examinations. Two patients demonstrated mild functional improvement determined by regular review at dialysis out patient clinics and patient self-reporting of symptoms: Patient 2 started extracorporeal photophoresis in January 2005 and patient 6 had spontaneous improvement of renal function to an eGFR of 49ml/min. Patient 5 started extracorporeal photophoresis in June 2006 and patient 12 received a functioning renal transplant in June

2006 but there was no detectable clinical improvement in their NSF at the end of the study period.

7.4 Discussion

A link between gadolinium-based contrast agents, in particular gadodiamide, and NSF was first postulated in January 2006, (77, 165) with further reports emerging thereafter (164, 166-169). At the time this study was completed there was no other case-control series examining this association or determining if there was a cumulative gadolinium dose-related relationship with the subsequent development of NSF.

These data align closely with those which have reported an association between gadolinium-enhanced MRI and the development of NSF in patients with ERF. These data suggest the association to be dose-dependent. No patient exposed to only a single standard dose of contrast agent (15 ml of 0.5 mol/l agent, approximately 0.1 mmol/kg) developed NSF. 1 in 130 (0.77%) of the overall ERF population developed NSF, however 1 in 32 (3.1%) of the ERF population exposed to gadolinium-enhanced MRI developed the condition. Mortality did not appear to differ significantly from that of the regular RRT population during this follow-up period.

When considering the international NSF registry, more than 95% of reported cases are said to have evidence of gadolinium exposure (170). Indeed, one patient in this study developed biopsy proven NSF without evidence of prior exposure to gadolinium. A clear

explanation of this remains elusive but such cases suggest that exposure to gadolinium may not be the sole causative feature to developing NSF. Similarly, in patients exposed to gadolinium, although the median time from gadolinium administration to the onset of NSF symptoms was 76 days, in four there was a period of months to years before clinical features developed. This lag time is longer than that reported by some other groups (77, 164-166, 171). Such findings suggest that gadolinium, although causally implicated, is not the only factor involved in NSF.

The combination of renal dysfunction and gadolinium exposure heighten the risk of developing NSF. Why these conditions predispose to NSF is uncertain. What we do know is that gadodiamide is distributed in the extracellular fluid and excreted unchanged by the kidneys with an elimination half-life in normal renal function of 80–100 minutes. Consequently 72 hours following gadolinium administration up to 95% has been excreted in individuals with normal renal function (176). The pharmacokinetics of gadolinium handling in established renal failure is poorly understood however, in patients with a glomerular filtration rate of 2-10 ml/min, not on dialysis, the elimination half-life of gadodiamide intravenous injection has been found to be approximately 34 hours. It follows that the lower excretion rate of gadolinium in established renal failure leads to a longer period of cumulative gadolinium exposure.

The type of gadolinium chelate used in contrast studies may also impact on the degree of free gadolinium exposure. Different gadolinium chelates demonstrate varying properties in terms of their structure (linear versus macrocyclic), electrochemistry (ionic versus non-ionic) and affinity towards binding proteins. Gadolinium-based contrast agents differ

significantly with respect to their propensity to transmetallate (release toxic free gadolinium from the chelate) and in terms of their kinetic and thermodynamic stability. Gadodiamide has a lower conditional stability constant compared to other gadolinium chelates despite the addition of excess chelate (169, 170, 173-175). This could be a plausible explanation for the especially pronounced association gadodiamide has with the development of NSF compared with other chelates. This remains a theoretical concept and the transmetallation theory has yet to be observed in pharmacokinetic studies of ERF patients. Indeed, it is not clear if alternative gadolinium-based agents are safer or if the preponderance of patients with NSF related to gadodiamide simply reflect its pattern of use.

Acidosis, anaemia and hypoalbuminaemia at the time of MRI exposure have been cited as potential cofactors in the development of NSF (77, 165, 169, 171). In this work no such associations were seen. Such data are very difficult to interpret in a study of this methodology as patients may undergo MR imaging to investigate co-morbid conditions including vascular disease or sepsis, which may be responsible for these abnormalities and potentially confound any association demonstrated.

How does gadolinium exposure relate to the development of NSF? Several recent studies using spectroscopy have demonstrated the presence of gadolinium in the skin of some, but not all, patients with NSF (169, 176-179). One autopsy study has demonstrated the deposition of gadolinium in the heart and vasculature of patients who died following a pathological diagnosis of NSF (180). A pathological study of biopsy proven NSF has demonstrated deposition of gadolinium in irregular small aggregates that adhered to collagen fibres within connective tissue in the dermis (179). Fibrocytes extracted from

these deposits in NSF patients have been demonstrated to synthesise excess levels of hyaluronan and collagen (181). These findings have led researchers to speculate that gadolinium deposited within the dermis is phagocytosed by tissue macrophages, triggering a localised cytokine cascade that promotes the release of pro-fibrotic mediators. These in-vitro studies, along with in-vivo studies such as this, may strengthen the association between gadolinium-based contrast agents and development of NSF; however have yet to prove causation.

Once NSF develops the clinical course appears varied. Some reports suggest a proportion of patients undergo gradual improvement in symptoms over time. This appears most pronounced in those who have subsequently undergone renal transplantation and thus gained a degree renal function. Complete recovery of NSF symptoms with ongoing stage V kidney disease has yet to be described. The majority appear to suffer heightened morbidity and impaired quality of life arising from musculoskeletal and other end-organ dysfunction. Reports to date suggest that approximately 5% of NSF patients have especially aggressive disease that may progress and rapidly result in premature death (138). Within this study follow-up times were short although mortality was not increased in patients with NSF when compared to their non-NSF counterparts.

No consistently successful treatment for NSF has emerged but extracorporeal photophoresis (ECP) has been advocated (151, 152, 182). ECP involves extracorporeal exposure of blood cells to photoactivated 8-methoxypsoralen, which are subsequently reinfused. How this exposure translates into a reduction in symptom burden is unknown. Some have postulated a role for ECP in modulating the activity of circulating fibrocytes however this has yet to be demonstrated in-vitro or in-vivo and thus remains a speculative

explanation (151). One patient in this cohort had undergone ECP with some improvement and a second commenced treatment just prior to the end of the study period. Randomised controlled trials of ECP treatment have yet to be performed in NSF patients.

The restoration of renal function, whether by treatment of the underlying renal disease or by renal transplantation, anecdotally appears to confer a degree of symptomatic relief. Up to 65% of injected gadodiamide may be eliminated during a single haemodialysis session whilst approximately 69% may be excreted over a 22-day period in patients on regular peritoneal dialysis (183). A more aggressive regime of peritoneal dialysis involving 10 to 15 exchanges per day has been shown to cause a 90% reduction in circulating gadolinium (182). Whilst these data suggest that an aggressive dialysis strategy following gadolinium exposure may be beneficial, one study describes three patients in whom NSF developed despite daily haemodialysis on each of the three days immediately following gadodiamide exposure (164). These data suggest such a strategy is therefore of limited benefit.

This report has several limitations, predominately arising through its retrospective observational design. Thorough patient review was conducted in an attempt to control for this although some issues could not be adequately addressed. The duration of each MRI scan was not recorded and examinations were performed on separate scanners, hence no comment can be made on the relationship between scan duration, magnetic field strength or MRI system type and development of NSF. The study population was restricted to those with CKD Stage V with no analysis of patients with acute renal failure or earlier stages of CKD. These populations are considerably greater in size than the stage V CKD population and thus establishing the risk of developing NSF in these groups is of considerable importance. As stipulated earlier, pathological confirmation of NSF is becoming

increasing important. Two patients in this study did not undergo skin biopsy and thus histological confirmation of the diagnosis could not be made. Conversely, the possibility that other patients with NSF were not diagnosed cannot be excluded, especially as awareness of the disease was limited 6 years ago. In comparison with the RRT population studied the NSF caseload was small in number and this limited the use of more detailed statistical analyses that could examine independence of association alongside other clinical, laboratory and demographic variables.

7.5 Conclusion

This study confirms the association between gadodiamide-enhanced MRI and the development of NSF in patients with Stage V CKD and undergoing RRT. These findings, together with those of other studies, suggest that gadodiamide should no longer be considered as without risk for use in patients with advanced renal failure (glomerular filtration rate < 15ml/min) and undergoing RRT.

Chapter 8

Discussion and Conclusions

8.1 Independent Risk Factors for Adverse Clinical Outcomes

The primary aim of this thesis was to perform a detailed evaluation of the risks to health conferred by aspects pertaining to the establishment and maintenance of haemodialysis vascular access in patients with renal failure.

The work detailed in chapter 2 was a retrospective analysis of bacteraemia and mortality in a cohort of haemodialysis patients with established renal failure that was representative of a typical UK chronic haemodialysis population. Despite a retrospective methodology in which a ‘snapshot’ of vascular access was related to outcomes over a subsequent 18-month period, use of a synthetic vascular access catheter was found to have an especially strong independent association with risk of bacteraemia and death compared with use of an arteriovenous fistula. These effects were found to be independent of known adverse prognostic factors such as advanced age, sex, diabetes, anaemia, and the other clinical and laboratory variables that were studied. The demonstration of such a relationship despite a methodology that was not sensitive to changes in vascular access type suggested that use of central venous catheters may have an especially strong association with adverse outcomes compared with other clinical and laboratory variables.

Both the non-tunnelled and tunnelled central venous catheter groups in this study demonstrated poorer outcomes than their arteriovenous fistula counterparts. Closer scrutiny of the two groups demonstrated those patients using tunnelled central venous catheters (TCVCs) to have been on renal replacement therapy (RRT) for a longer period of time compared to patients with non-tunnelled central venous catheters (NTCVCs). Analysis of the individual case-mix suggested this to be a product of patients whose

peripheral vasculature had been exhausted of attempts to create an arteriovenous fistula or graft. Indeed, the demographics and co-morbidities of the TCVC group and arteriovenous fistula (AVF) group were found to be very similar and thus the conclusion that adverse outcomes are independently associated with TCVC use when compared to AVFs would appear to be valid. When considering the NTCVC group, it was evident that this consisted of a higher proportion of patients who had commenced RRT for established renal failure within three months of study entry and in this setting the co-morbidities that often arise during this period may have contributed to the difference in clinical outcomes seen in this particular group when compared to the TCVC and AVF groups.

Whilst this study suggested that central venous catheter use was associated with poorest outcomes, the different characteristics expressed by the TCVC and NTCVC groups made direct comparison limited. What was not clear was the degree to which the different event rates expressed between groups was a function of the clinical characteristics of those undergoing a specific type of catheter insertion, or a function of the type of catheter insertion procedure itself. The lack of detailed data on comorbidity, other than the data we included regarding age, duration on RRT and diabetes compounded this problem.

To address these limitations, a prospective study of catheter insertion procedures was designed. This methodology allowed real-time measurement of a broader number of comorbidity markers including Charlson score – a validated means of comorbidity scoring – and was sensitive to real-time changes in vascular access method. The ensuing data, presented in chapter 3, demonstrate the significant differences between tunnelled and non-tunnelled central venous haemodialysis catheter insertion procedures in terms of rates of catheter-related bacteraemia and another clinically significant outcome in catheter use,

catheter failure due to poor and unsustainable haemodialysis blood flow. Patients with TCVCs demonstrated consistently better outcomes than those using NTCVCs, independent of the wide range of clinical and laboratory characteristics that were recorded. Within the NTCVC groups a clear hierarchy of independent risk association was seen with de-novo internal jugular catheterisation being the best performing NTCVC procedure and guidewire catheter exchange in the femoral veins being the poorest performing NTCVC procedure. Again, these outcomes were independent of other important clinical and laboratory variables.

These data bring greater clarity to the interesting debate of whether such adverse events develop as a function of the characteristics of the catheterisation procedure or the characteristics of the patient receiving the catheter. It appears that the catheterisation procedure, in terms of tunnelling and site of insertion, contributes independently and most significantly to these important clinical outcomes. The demonstrated hierarchy of risk association within NTCVC insertion procedures is of considerable clinical relevance; this should help guide clinical practice and could even be used as an evidence-based foundation on which clinical guidelines for central venous catheterisation for haemodialysis may be built.

Whilst the study provided as detailed an account of independent risk association that most prospective observational studies could offer, the definitive answer to these questions can still only be achieved by randomised controlled trials. Until such studies are conducted and designed to account for variables such as the number of previous vascular access procedures and standard baseline clinical and laboratory demographics, our understanding

of vascular access will remain limited to such observational work. Furthermore, this study was limited to outcomes that relate to infection and thrombosis, other outcomes such as mortality, subsequent rates of conversion onto a functioning AVF or graft and subsequent sustainability of that AVF or graft remain of considerable interest. Mapping the vascular access journey for a population of haemodialysis patients and relating this to these outcomes may identify the key junctures in which haemodialysis patients either follow a path of optimally safe, long-term, vascular access establishment, or may deviate down a route of repeated short lifespan vascular access methods, high complication rates and early death. By identifying these points on a population level, interventions designed to prevent adverse outcome could be applied and outcomes improved. It is these areas in which there remains a pressing need for further in-depth study.

8.2 Optimising Catheter Performance with Lock Solutions

Once established, the successful maintenance of patent central venous catheters for haemodialysis is of critical importance. Historically the most common complication that limited catheter lifespan was catheter thrombosis. Unfractionated heparin has become almost universal in its use across the world as a catheter-locking agent by preventing intraluminal catheter thrombosis due to its proven anticoagulant effect. Catheter-locking practices have, however, evolved over recent years with an increase in the use of combined solutions that both limit thrombosis and lower risk of catheter-related bacteraemia by preventing and reducing bacterial biofilm. Whilst the benefits of catheter-locking with combined anticoagulant and antimicrobial solutions are becoming increasingly well

documented, the potential limitations or pitfalls of these techniques have not been fully explored. The work described in chapters 4 and 5 examine the potential for catheter lock solutions to leak into the systemic circulation in patients, and examine how combinations of anti-coagulant and antimicrobial may alter their antimicrobial properties in vitro.

The work described in chapter 4 successfully demonstrated that use of heparin as a catheter-locking agent in haemodialysis patients is associated with a significant degree of systemic heparinisation as demonstrated by prolongation of the activated partial thromboplastin time (APTT). Systemic heparinisation occurred in both of the groups studied, either heparin 1000iu/mL or 5000iu/mL, despite only instilling a volume of heparin equal to the internal catheter luminal volume stated by the catheter manufacturer. Use of heparin 1000iu/mL conferred a significantly lower risk of systemic heparinisation than heparin 5000iu/mL. There were, however, no instances of haemorrhage in either group over the course of the study, albeit within a relatively small cohort with a relatively short duration of follow-up. The lack of any significant difference between groups with regard to the secondary outcome measures of catheter-related bacteraemia and catheter failure due to poor haemodialysis blood flow is of limited value due to the small sample size, the lack of power to examine these outcomes, the heterogeneity of non-tunnelled catheter insertion sites and the relatively short duration of follow-up.

Whilst these findings are of particular clinical interest routine clinical practice cannot be greatly shaped by this study. In specific instances however, such as when performing haemodialysis in patients with a significant bleeding risk, use of a lower concentration of heparin solution may be conducted knowing that this will lead to less systemic

heparinisation between dialysis sessions and thus would be an appropriate additional measure to undertake.

This study does raise other important clinical points, especially when considering the utility of combined anticoagulant-antimicrobial catheter lock solutions. The concept of occult leakage of catheter lock solution into the systemic circulation may promote an environment in which antimicrobial resistance could be allowed to flourish. Within the catheter lumen the concentration of antimicrobial is invariably very high and contained within a low volume of only a few millilitres – few, if any, susceptible microbes could survive in such an environment let alone develop antibiotic resistance. If the solution leaks into the systemic circulation the volume of distribution for that antimicrobial increases considerably. This dilution may produce systemic levels of antimicrobial that are of sufficiently low concentration to promote the preferential growth of antimicrobial-resistant microbes. When considering a population of haemodialysis patients the emergence of antimicrobial resistance could lead to significant morbidity and mortality. Through the same mechanism other adverse effects, such as ototoxicity with gentamicin, may become increasingly expressed by a dialysis population through continuous cumulative exposure to an antimicrobial. It must be emphasised, however, that such limitations only realistically apply to those in whom concurrent systemic delivery of the antimicrobial is not taking place. As such, these limitations would only appear relevant to those undergoing primary prevention of bacteraemia as opposed to those with a catheter-related bacteraemia who are reliant upon catheter salvage i.e. use of catheter locking as an adjunctive secondary prevention strategy. Indeed, how the addition of catheter lock solutions to standard catheter salvage practice may affect outcome is another important question which remains to be fully evaluated.

This study highlights both the need for detailed outcome data when evaluating combined catheter lock solutions, and the need for long-term follow-up of the patient populations in which these techniques are trialled. Whilst these uncertainties persist there remains a pressing need for a means of maintaining catheter patency and limiting bacteraemic events in haemodialysis patients with the minimum of adverse events. Optimal catheter lock solution composition, technique of delivery and circumstances of use remain to be fully clarified and shall require continued study.

It is also important to determine whether any interaction between constituent anticoagulant and antimicrobial solutions may exist and, if so, how this may affect overall performance. The work detailed in chapter 5 aimed to elicit whether any such interaction occurs between heparin and vancomycin on planktonic and biofilm embedded staphylococci.

These in-vitro data demonstrated that heparin alone may independently heighten or suppress bacterial growth, depending upon the staphylococcus studied. Across a range of vancomycin concentrations, the addition of heparin appeared to have the effect of lowering the MIC. Similar patterns were seen when considering biofilm embedded staphylococci. This suggests that heparin could modulate bacterial growth when combined with the antimicrobial vancomycin. Repeating these analyses with viability counts would be a way of testing this hypothesis by demonstrating the killing effect of the lock solution.

Whilst of pharmacokinetic interest, these in-vitro findings were elicited in a small study of seven laboratory-grown staphylococcal isolates, albeit known biofilm producers and thus potential pathogens for catheter-related bacteraemia. An evaluation of staphylococcal

isolates taken from cases of proven staphylococcal catheter-related bacteraemia would have greater clinical validity. The relatively small number of duplicate experiments and sub-optimal quality of the biofilm modelling are all aspects of the methodology that would require further refinement before these conclusions could be meaningfully advanced.

These findings are, however, of significant clinical interest. Standard catheter practice in many renal units involves catheter locking with heparin alone. Whilst this may suppress growth of staphylococcal sub-species, others may experience heightened growth. When considering combined antimicrobial-anticoagulant solutions excellent intraluminal suppression of biofilm may be achieved but, with occult leakage, may occur at the expense of poor suppression of planktonic bacterial grown in the bloodstream and biofilm production on the outside of the catheter containing increasingly resistant populations of organisms and perhaps accelerated by the presence of heparin.

These theoretical limitations merit scrutiny. This may be achieved by further laboratory study looking at intraluminal and extraluminal biofilm growth under differing antimicrobial-anticoagulant conditions. On a clinical level, the role of extraluminal catheter biofilm versus intraluminal catheter biofilm with regard to risk of metastatic bacterial infection such as endocarditis and osteomyelitis remains unclarified and the presence of secondary metastatic infection would be a useful clinical endpoint when considering the clinical utility of combined antimicrobial-anticoagulant catheter lock solutions in vivo. Randomised controlled evaluation of combined antimicrobial-anticoagulant solutions using bacteraemia, resistance patterns, adverse events, catheter thrombosis and mortality as outcomes over both short and long-term periods of follow-up

could further tease out the relative advantages and disadvantages of these practices. By performing such studies optimal catheter-locking practice with combined antimicrobial-anticoagulant solutions may be determined.

8.3 Vascular Imaging in Haemodialysis Patients

Patients on RRT for many months or years are exposed to multiple vascular access procedures. As time on RRT progresses vascular access complications may accumulate, impairing the function of existing access and limiting the availability of sites for future access. It is under these circumstances that imaging of the central veins is used to determine appropriate access points to the vascular tree with the aim of linking suitable venous architecture with optimal access function. Contrast enhanced magnetic resonance venography (CE-MRV) had become increasingly used over recent years due to its proven speed, accuracy and reproducibility in the detection and evaluation of central venous thrombus and stenosis (54). Few had, however, formally quantified its clinical applicability and thus the work detailed in Chapter 6 was designed, studying all CE-MRV examinations undertaken in haemodialysis patients within Glasgow Royal Infirmary Renal Unit.

This work described the abnormalities found on CE-MRV and the subsequent clinical course followed by the patients in whom CE-MRV imaging was undertaken. The methodology used was observational, retrospective and in a select group of problematic patients and thus has to be taken carefully in this context. Despite these limitations several

important themes were demonstrated. CE-MRV gave a definitive assessment of venous patency that had proven difficult to quantify by clinical or other radiological means. Abnormalities were found in 74% of CE-MRVs with patients in whom an abnormality was found being significantly more likely to have successful vascular access establishment in the 6 months following scanning than those with a normal CE-MRV. A positive association between the number of previous arteriovenous fistulae or synthetic graft procedures and the development of central venous stenosis was demonstrated. Negative findings on CE-MRV also proved useful with several patients.

These advantages however have had to be tempered with the emergent finding that four patients within the cohort developed nephrogenic systemic fibrosis (NSF) following CE-MRV. This coincided with the emergence of gadolinium contrast agents as a potential causative agent in the pathogenesis of NSF in published literature (77). The serious nature of the condition effectively ended the mainstream usage of CE-MRV in RRT patients and highlighted the benefit of post-marketing surveillance when considering the use of medicines/contrast media extending into specific patient sub-groups. Given the uncertainties surrounding the aetiology and pathogenesis of NSF, the work described in chapter 7 was designed. On completion of this study there was no other case-control series examining this association or determining if there was a cumulative gadolinium dose-related relationship with the subsequent development of NSF.

The findings of this study supported the reported association between gadolinium-enhanced MRI and development of NSF in patients with ERF. The relationship appeared dose related although mortality appeared to be unaffected when compared with the non-NSF cohort studied. One patient in the study developed biopsy proven NSF without exposure to

gadolinium and in several instances the median time from gadolinium administration to the onset of NSF symptoms was relatively prolonged. These findings suggest that gadolinium, although causally implicated, was not the only factor involved in the development of NSF.

This study provided a significant step forward in our understanding of the association between gadolinium and NSF development. Importantly, however, this study reported association, not causation and thus only provides a basis on which pathogenetic mechanisms can be hypothesised before being tested by in-vitro and in-vivo assessment. Several key areas require scrutiny.

Firstly, the sequence of events linking gadolinium exposure, renal dysfunction and the development of a pro-fibrotic response requires elucidation. A number of in-vitro studies have highlighted the prolonged excretion of gadolinium in renal failure, the deposition of gadolinium in the dermis and the pro-fibrotic responses that arise from local tissue injury in association with gadolinium deposition. These in-vitro data need to be pursued further before a solid, tangible pathogenetic mechanism can be put forward. Secondly, can modulation of these variables be undertaken that not-only reduces but obviates the risk of developing NSF? The comparison of cyclic versus linear gadolinium chelates, effective renal replacement strategies that limit exposure/heighten excretion of the chelate and the generation of antifibrotic targets all merit detailed study. Thirdly, what treatment strategies are effective in limiting the morbidity, progression and mortality associated with established NSF? Few, if any of the therapeutic options reported have more than anecdotal support to their use. Given the rarity of the condition and the assumption that the case incidence will decline following the withdrawal of CE-MRV in stage V CKD, this question may never be fully answered. Whilst these issues remain unresolved the benefits that CE-

MRV provided cannot go unrecognised and thus there remains a significant need to pursue other methods of imaging the vascular tree in which such reproducible image quality is obtained without any resultant adverse effect.

8.4 Conclusions

Successful vascular access provision is the bedrock on which successful haemodialysis is built. The considerable expansion of haemodialysis worldwide has been built on a template of simple vascular access rules – fistula first, catheters when in crisis. Within these rules it is clear that no method of vascular access remains risk free. With the increasing provision of haemodialysis to aging, comorbid populations these rules require refinement and each facet of access provision requires close scrutiny.

This thesis has provided a detailed evaluation of the risks to health conferred by some of the key elements of haemodialysis vascular access provision and maintenance. The clear hierarchy of adverse risk across vascular access types, the spectrum of independent risk association expressed across the range of catheter insertion procedures, the varied considerations required when assessing catheter locking strategies and the advantages and disadvantages of imaging the vascular tree are important themes which have been explored, developed and clarified within this thesis. Many questions have been answered and yet in many instances another set of important questions have arisen. It is of considerable importance that many of the issues described in this thesis continue to be explored, solutions developed and outcomes improved in this especially large population of

vulnerable patients. Small changes applied across a large, vulnerable, population can make a considerable change to outcome. The safe and effective provision of haemodialysis vascular access therefore remains an area in which considerable improvements in the health of renal patients may be made in the future.

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